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ASCARIASIS IN HORSES

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Ascariasis in horses seems to be common in Saskatchewan. Dr. B. H. Ransom and the writer (1918) performed autopsies on twenty-two horses in various parts of the province and found that twelve of these harbored *Ascaris equorum*. During the past season a number of infected animals have been found in the course of experiments on swamp fever. In 1923 seven cases of bronchitis and pneumonia in young foals developed at the University Farm at Saskatoon. A tentative diagnosis of ascariasis was made, but it was not possible to confirm it until later in the year, when the university veterinarian, Dr. Wright, treated these same animals with carbon bisulphide and oil of chenopodium. Several of the colts passed large numbers of ascarids and one of them over 300. Two of the colts died soon after the outbreak started, another lingered on but died some months later, the remainder coughed and ran at the nose but eventually recovered. The fact that no other horses at the university were suffering from colds at the time the colts became ill was the reason for the tentative diagnosis of ascariasis.

METHOD OF INFECTION IN FOALS

Ransom and his associates have shown the various ways in which pigs pick up Ascaris eggs. But in the case of horses there must inevitably be differences. Foals come into the world under much the same surroundings as pigs—that is to say, the mare is put to foal in a loose box. It is here no doubt that the young foal picks up many eggs—not so much during the act of suckling as is the case in pigs—but when nosing about among the litter on the floor of the box stall. Colts have a habit often of nibbling at manure, especially in the barn yard where the piles of dung might easily contain ripe eggs. Out in the fields it is not so probable that foals would pick up eggs—except from the afore-mentioned habit of nibbling at piles of droppings. As the eggs do not hatch on the ground they would tend to gravitate under the vegetation, helped by wind and rain, and there remain harmless. It

appears likely thus that the box stall and the stable are the places to look to for the source of the infection. Thus the season of 1923, when the foals became infected, was wet, and the foals and their dams spent a good part of the early season in the stables. 1924 was a dry year, and they were not brought in as much; added to this the stables had been newly painted, so the 1924 crop of foals escaped infection so far as is known. The prevention of ascariasis would accordingly seem to be largely a matter of stable cleanliness.

It is believed by many that immunity to or tolerance of worms and other parasites increases with the age of the host, and that the mere fact of an animal getting older enables it to tolerate or resist them to a greater degree. An attempt will be made by the writer to explain some of his own observations on animals and to mention some of the views of other writers which bear on the subject. The damage parasitic worms produce comes under two headings, first mechanical, and second, that caused by their excretions or secretions. The mechanical part will not be dealt with here beyond pointing out that organs or tissues which have been invaded by parasites may have had their functions upset by nerve irritation caused by injury, or by hemorrhages and other means.

The products given off by worms cause their host to become sensitive to them. Many workers seem to agree on this point. The balance between these substances and the anti-substances which neutralize them must be very evenly distributed just as they are in some of the bacterial diseases. There is evidence that this is correct in that adult animals are commonly able to maintain their good health while harboring many parasites. Young animals not having yet developed this power of tolerance suffer severely from their effects. The dangerous time for young animals appears to be, especially in the case of Ascaris, when the migration takes place through the body. The passage of the larvae through the liver brings on a degeneration of the liver cells which must have a serious effect on nutrition. In the lungs edema follows their migration, which in turn may result in more serious disturbances. In the older animals, which on autopsy are found to be carriers of Ascaris, the liver and the lung lesions, except for old scars, etc., may be entirely absent. This seems to point to the fact that once the worms have completed their migration and have settled in the intestine they have reached a sort of neutral position as regards the health of their host. It is still possible that the absorption of their dejecta in the intestine might keep up the irritation in sensitized tissues such as the lungs.

To sum up, the writer believes that the result of a first invasion of worms in an unresisting host is to stimulate the production of both antisubstances and eosinophiles to neutralize their cast off products, and that in addition to this there must be a third substance which is antago-

nistic to the worms themselves; this substance which paralyzes or kills them he believes is secreted by the eosinophiles that are found surrounding the dead worms in the tissues. This theory is supported by the fact that he has seen cases where only a percentage of the invading parasites died surrounded by eosinophiles, whereas in the same host other worms evaded the eosinophiles either because of their rapid movements, or because owing to their numbers the eosinophiles were not numerous enough to cope with them.

SENSITIZATION

A suggestion is made that the edema of the lung following Ascaris invasion may be, in some cases, in part or wholly due to sensitization. This would be most likely to happen when two or more invasions of parasites have occurred in the same host. In two cases in colts the lungs appeared to be free from bacteria as all culture tubes inoculated from them remained sterile, yet parts of the lungs were edematous. In experiments made with other parasites the writer has noticed that tissues which have once been attacked by parasites become sensitive (local sensitization) and react again when the juices of the same parasites are injected into the general circulation.

OPINIONS OF VARIOUS EXPERIMENTERS

Ransom (1924) says: "Its significance [sensitization] in relation to the toxic action of Ascaris in cases of infestation with the parasite remains to be determined." Weinberg and Julien (1911) say: "Ces faits nous autorisent, croyons nous, a penser que les chevaux infestés par un certain nombre de parasites [Ascarids] s'immunisent petit a petit contre l'action des produits secretés par ces parasites." Roubaud and Perard (1924) say: "Les auteurs estiment qu'il s'agit d'un fait d'immunisation consecutif a une ou plusieurs atteintes subies" [Refers to Hypoderma.] Hadwen and Fulton (1924) state: "It seems, therefore, that the immunity that develops in the older cattle is effective in the prevention of warbles."

Fairley (1919), according to Agersborg (1924) "showed that there is a definite relationship between the cellulo-humeral response in experimentally infected monkeys (infected with *Bilharzia haematobia* and *Bilharzia mansoni*), and the prognosis. In hyperinfected monkeys dying within a few weeks he found that there was a constant leukopenia, absence of eosinophilia, and a negative complement fixation reaction. In monkeys surviving the sixth week of infection there was constantly present an eosinophile leukocytosis associated with a positive serological reaction." Yokogawa (1923) says: "We do not know how to ascertain the essence of the virus of the Ascaris larvae, but as the accumulation of eosinophile cells is conspicuous wherever they happen to be

found, it is easy to infer their virulence." Höppli (1923) infected a large number of small animals with embryonated eggs of *A. lumbricoides* and also with Belascaris and Toxascaris. He found that the tissues reacted only slightly against the first invading parasites, but a few days later he found that the neutrophiles, eosinophiles, and mononucleurs appeared in groups, in some cases surrounding the parasites. In the case of *A. lumbricoides* there was little tendency on the part of the tissues and cells to encapsulate the larvae. On the contrary he found many encapsulated larvae of Belascaris in the livers of dogs. Höppli makes no mention of the ages of the animals he was infecting, and it would seem likely that in the Belascaris experiments he was dealing, in some cases at least, with dogs which had developed a certain amount of immunity to ascarids. Höppli himself thought that there might be some secretion which would cause a tendency to encapsulation and says: "Eine sehr wesentliche Ursache für die Knotchenbildung bietet auch die, allerdings noch unvollkommen untersuchte Absonderung spezifischer Stoffe." Höppli confirms Ransom's observations on the migration of the larvae via the blood stream, and denies Yoshida's views.

THE EOSINOPHILE CELL AS A DESTROYER OF PARASITES

In the experiments on Ascaris described below it has been shown that the percentage of eosinophiles in the general circulation lowers after an infective dose of eggs has been given and that it rises again soon after the worms have finished their migration through the tissues. The writer in cooperation with Ransom (1919) showed that injections of various worm juices in horses produced this same lowering and elevation of the eosinophile count. The writer in 1916, 1918, 1922 and 1924 also drew attention to the fact that the eosinophile cell was a most important factor in the prevention of infestation with both Hypoderma and Oedemagena. Vallillo (1919) says that the increase of eosinophiles is not a constant symptom perhaps because the eosinophiles come out of the blood vessels toward the tissues. S. Sterling-Okuniewski (1924) says: "Dans la trichinose, il y a une éosinophilémie, celle-ci pouvant etre légère (9 à 14 p. 100) dans les cas sérieux, ou très élevée (50 p. 100) dans les cas légers."

OBSERVATIONS ON EOSINOPHILES MADE BY THE WRITER AT DIFFERENT TIMES AND WITH VARIOUS PARASITES

Local reactions in which eosinophiles are numerous are uncommon in young animals and are common in older animals. In the case of the penetration of the skin of cattle by Hypoderma, the reactions become more and more severe as the season advances. That eosinophiles actually do neutralize verminous toxins is supported by the fact that these cells

will traverse intact mucous membranes when these have been bathed in verminous juices. Eosinophiles surround worms of all sorts when they become stationary in the tissues. Eosinophiles take on a phagocytic rôle when bacteria are bathed in verminous juices. (This was first shown by Weinberg and Seguin, 1914.) Eosinophiles act in a local or systemic way, when large or small amounts of worm juices are injected into the body. Eosinophiles do not appear at once in the case of local injections; an edematous swelling comes first which indicates that the body fluids, also cells such as the neutrophiles and mononuclears, play a part in the neutralization of the offending substance. The slowness in the production of the eosinophiles does not indicate that they are unnecessary for the successful neutralization of toxin or for the destruction of parasites. It may be interpreted to mean that the cells are only produced as occasion demands. There is a great difference between the neutrophile cell and the eosinophile, for instance, the former cell is in constant demand to act in the defense against bacteria—these however, are constantly attacking animals while worms do so irregularly. The percentage of eosinophiles in the circulation may be low or the cells absent altogether in severe cases of parasitism. In such cases the eosinophiles have been drawn out of the circulation and are to be found in large numbers around about the parasites themselves. When the proper percentage returns into the general circulation it means that the parasites have been overcome and that there is a surplus of eosinophiles. With regard to worms situated in such places as the lumen of the bowel or in the cavities of the body, this may not hold true, but this refers to parasites within the tissues.

Experiments with *Ascaris equorum*. Experiment I (on a young sucking colt 12 days old)

Day 1 (May 20, 1923) Colt given a petri plate of Ascaris eggs.
Day 7 Colt breathing hard and seemed distressed.
Day 7-13 Colt distinctly unwell but no cough.
Day 13 Given second dose of Ascaris eggs.
Day 16 Colt coughs a little. Temperature 102.5.
Day 16-20 Coughs continuously.
Day 20 Colt killed. *Post mortem:* Liver badly spotted. Lungs slightly affected in anterior lobes. Numerous Ascaris larvae found in air passages which contained a considerable amount of mucus. In the duodenum a number of ascarid larvae about 2 mm. in length.

COMMENTS ON EXPERIMENT I

The colt developed a husky cough after the administration of the second dose of Ascaris eggs. Larvae were recovered from the lungs in great numbers. It is probable that the time it takes *A. equorum* larvae to make their journey from the intestines to the lungs and back again is similar to that of *A. lumbricoides* in the pig, as fairly well developed larvae were found in the duodenum twenty days after the first dose was given and seven days after the second dose.

Experiment II (on a 4-5 months old colt naturally infected with Ascarids)

Day 1 A few Ascaris eggs found in feces.
Day 2 Colt given drench of Ascaris eggs.
Day 20-24 Slight cough.
Day 32 Differential leucocyte count. Monos. 62.2, poly 24.8, eosins 12.5, mast 0.5.
Day 33 Given second dose of Ascaris eggs.
Day 35, differential leucocyte count. Monos, 58; poly, 35.5; eosins, 5.5; mast, 1.
Day 38, differential leucocyte count. Monos, 69.5; poly, 29; eosins, 1.5; mast, 0.
Day 45, differential leucocyte count. Monos, 48.5; poly, 44; eosins, 4.5; mast, 0.
Day, 50, differential leucocyte count. Monos, 57; poly, 35.33; eosins, 5.66; mast, 2.0.
Day 52, differential leucocyte count. Monos, 47.5; poly, 41; eosins, 11; mast, 0.5.
Day 54, differential leucocyte count. Monos, 34.7; poly, 49.7; eosins, 13.3; mast, 2.3.

Temperatures. Taken daily during experiment. No rise over 102.5.

Day 54 Colt killed. *Post Mortem:* Animal pot bellied, slight anemia, lungs spotted with translucent white nodules. Liver spotted and degenerated. Heart, endocarditis. Ascarids in intestine, 19 male and female and 7 smaller worms. 183 small larvae about 2-3 c.m. long and 203 larvae about 1.5 c.m. long.

Cultures. Inoculations made from liver and lungs remained sterile.

COMMENTS ON EXPERIMENT II

This animal was beyond the age for severe symptoms to set in after the administration of eggs. The only effect noticed was a slight transient cough. This case is the most interesting of the three, as there was a well marked disturbance in his blood when the second dose of eggs was given. It seems evident that the diminution of the eosinophiles in the general circulation was due to their being attracted to the liver and lungs to repel the new invasion of larvae. They again appeared in the circulation—even in increasing numbers 21 days later. In sections made from the lung and liver (colts Nos. 1 and 2) eosinophiles were found in large numbers. In the lungs many larvae had been destroyed in their migration and small pearly white nodules were plentiful just under the pleural covering. In the liver no larvae were demonstrated in sections.

PATHOLOGICAL EFFECT OF LARVAE MIGRATING THROUGH THE LIVER

The liver cells showed a grey granular degeneration and many of them stained badly, especially the nuclei. Surrounding Glisson's capsule eosinophiles were grouped like a ring, in many cases, and they were also to be found under the capsule of the liver. It seems evident that many larvae instead of traveling by the circulation follow the connective tissues along the vessels. The eosinophiles were not scattered uniformly throughout the liver, so that the groups of eosinophiles round about the vessels made a striking picture. The tissues surrounding the vessels

were absolutely free from eosinophiles but in those cases where the eosinophiles encircled them, numbers could be found actually in the coats of the vessels. Possibly these cells were passing through the walls of the vessels to join the cells outside. In the blood itself inside the vessels very few eosinophiles were noted. From the above it seems certain that the eosinophiles only collected round about the hepatic vessels and ducts in those places where the worms had actually travelled. Ransom and Cram (1921) tapped the blood vessels in different parts of the body and found the larvae inside them. Their observations give absolute proof that the larvae—no doubt the majority—travel by way of the vessels.[1]

The writer's observations, he believes, show however that many larvae follow the connective tissues along the vessels. The cellular reaction shows that such is the case because it is not likely that the eosinophiles would group themselves round vessels through which the larvae had merely passed on their way to the heart and lungs. Stewart (1917, quoted by Ransom) thought that one likely route followed by the larvae was along the capillaries of the liver between the degenerated columns of liver cells. However the degeneration of the cells could not have occurred until after the passage of the first batch of invading larvae.

The part played by the eosinophiles in the neutralization of verminous toxins and in the destruction of larvae would necessarily be after the first invasion; this view entirely fits the case with regard to the progressive development of immunity against ascarids, and is borne out by the differential counts given above. The mechanism of defense by the eosinophiles is no doubt imperfect in the domesticated animals, because of the sudden unexpected number of worms invading the body. If the invasion occurred little by little it is probable that the immunity would keep pace with the invaders. An example of an unresisting host to parasitic infection is well illustrated by the case of a calf attacked by warble larvae. The period of attack is short and the invasion often large.

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1. In a personal communication Ransom says: "If the larvae follow the connective tissues along the vessels they ought occasionally at least to be found there, especially since their progress would probably be slow along such a path." This objection is no doubt valid with regard to the migration of the larvae through the liver, as in all the sections made no dead larvae were encountered. In the case of the smaller vessels of the liver where by mechanical injury or through their secretions, the larvae could cause actual damage, the cellular reaction might be considered as an attempt at repair. But in the larger vessels the onward movement of the larvae would be much quicker and not likely to damage the walls. In the lung sections numerous dead larvae were found surrounded by a zone of eosinophiles. However, where long streaks of eosinophiles were noted between the lobes, the reaction could be interpreted as consecutive to the passage of the larvae."

PATHOLOGICAL EFFECT OF LARVAE IN THE LUNG

A line of eosinophiles ran under the covering of the lung, and other lines could be traced following the bands of connective tissues between the lobes. Whenever a larva had been successfully surrounded by eosinophiles, the dead larva could be seen in the center of the ring. In the surrounding zone the material appeared to be of an amorphous nature in which there were many eosinophiles and innumerable eosinophilic granules.

Experiment III. (Colt 12 days old given a petri plate of *Ascaris equorum* eggs)

Day 1 (Oct. 9, 1924). Temperature 102.3
Day 5 Temperature 104.2
Day 9 Cough began and continued until day 140
Day 17 Temperature 105
Day 18 Temperature 104.2
Day 19 Temperature 104
Day 21 Temperature 105.2
Day 22 Temperature 105.2
Day 23 Temperature 105.1
Day 24 Temperature 104.2
Day 25 Temperature 104.3
Day 26 Temperature 105.2
Day 27 Temperature 103.4
Day 53 Temperature 104
Day 54 Temperature 104
Day 55 Temperature 103.4

Day 71 Temperature 105.3. No Ascaris eggs in feces. Differential leucocyte count: Poly, 57; monos, 43. No eosinophiles found though hundreds of cells counted.

Day 71-190 No temperature over 103.

Day 138 Numerous Ascaris eggs in feces.

Day 145 Differential leucocyte count: Mono, 65; poly, 31; eosins, 3.7; mast, 0.3.

Day 154 Differential leucocyte count: Mono, 77.3; poly, 19.7; eosins, 2.5; mast, 0.5.

Day 190 Differential leucocyte count: Mono, 79.5; poly, 18.3; eosins, 2.2; mast, 0. Colt has not developed well, his mucous membranes are pale and his abdomen slightly pendulous.

Day 191 Colt killed. Just before the colt was killed it was found that his temperature had risen to 105. On autopsy nothing was found to justify this rise — which may have no significance as regards Ascaris.

Post Mortem: Body fairly well nourished. Mucous membranes pale. The abdominal cavity contained about 200 c c. of fluid. This had been in much larger amount previously, as all the viscera were covered wth short fibrinous threads (¼-½ inch). The liver was enlarged and spotted and some small calcified tracks were noted. Heart, slight endocarditis. Lungs spotted; a few small hepatized areas in anterior lobes. On the dorsal surfaces of the main lobes the blood vessels showed small varicosities and portions of the lung were spotted and congested. The small intestine had a hard and thickened feel, it contained much white mucus. Two hundred and sixty-seven adult living ascarids were taken throughout its length, measuring on an average 24 cm. long. In the cecum and colon 29 dead ascarids were found. In the cecum 5 adult *Strongylus vulgaris* were collected and in the stomach 27 bots (*Gastrophilus intestinalis*). The colt had been kept in a loose box from birth (September 28th) and had never been taken out of the stable until after the winter set in in November— hence the colt had acquired Strongyles and bots from its mother in the stable. Smears made from liver and lungs showed a few eosinophils and some eosinophilic granules. Cultures made from the liver and lungs remained sterile. In the intestinal mucus eosinophiles were scarce; this also was the case in Colt II.

COMMENTS ON EXPERIMENT III

Colt III, like Colt I, was young and free from worms when the Ascaris eggs were given. Nine days later the colt began to cough. Soon after this his temperature rose and on the 17th day reached

105 F. It remained high until the 26th day when it fell a little. On the 53rd day it rose to 104 and on the 71st day went up to 105.3. After the 71st day no temperatures of over 103 were recorded up to the 190th day. It seems probable that the high temperatures were the result of infection following the migration of the larvae, however, there does not seem to have been any lengthy infection either in the liver or lungs as cultures made from them remained sterile.

The differential counts show that there was an absence of eosinophiles in the circulation on the 71st day, but on day 145 when the colt was over four months old the eosinophiles were returning though not in great numbers and they were also present on days 150 and 190. The total absence and then return of the eosinophiles suggests that the colt was beginning to resist ascarids, and it seems likely that if one or more doses of eggs had been given the eosinophiles would have increased as in the case of Colt II. In this connection it is interesting to note that a number of dead ascarids were being passed out by the colt. The exact significance of the cause of death in the worms could not be ascertained.

SUMMARY OF EXPERIMENTS

Three colts, two new born and one older, were dosed with embryonated *Ascaris equorum* eggs. In the two younger animals a cough developed 16 and 9 days after the eggs were given. The older animal only developed a slight cold after 20 days. Ascaris larvae were recovered from the air tubes. The eosinophiles in the circulation became more numerous after a second dose of eggs.

In Colt III no eosinophiles could be found in the blood stream 71 days after the infective dose was given, but they appeared on the 145th day. This is suggestive of the slow development of immunity. In Colt II an eosinophilia was stimulated by a second dose of eggs.

Sections of the liver showed a granular degeneration, and accumulations of eosinophiles around Glisson's capsule. In the lung the eosinophiles were grouped under the pleura and between the lobes. This eosinophilia suggests that many of the larvae migrate along the connective tissues outside the vessels. Dead larvae were found in the lungs surrounded by eosinophiles.

The theory is advanced that immunity to ascarids is stimulated and increased by repeated attacks of these parasites. It is also suggested that in addition to the production of anti-substances to neutralize the cast off products of the worms, there is another substance secreted by the eosinophiles which is detrimental to the worms themselves.

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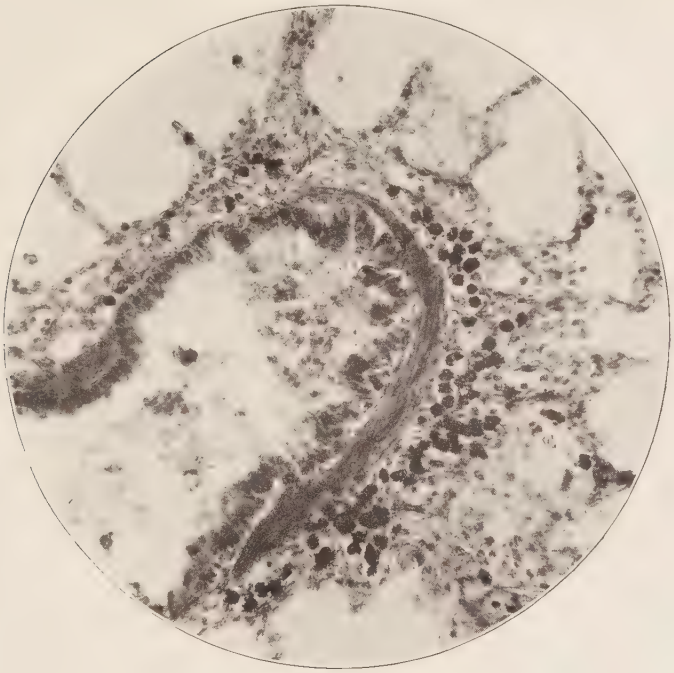

Figure 1

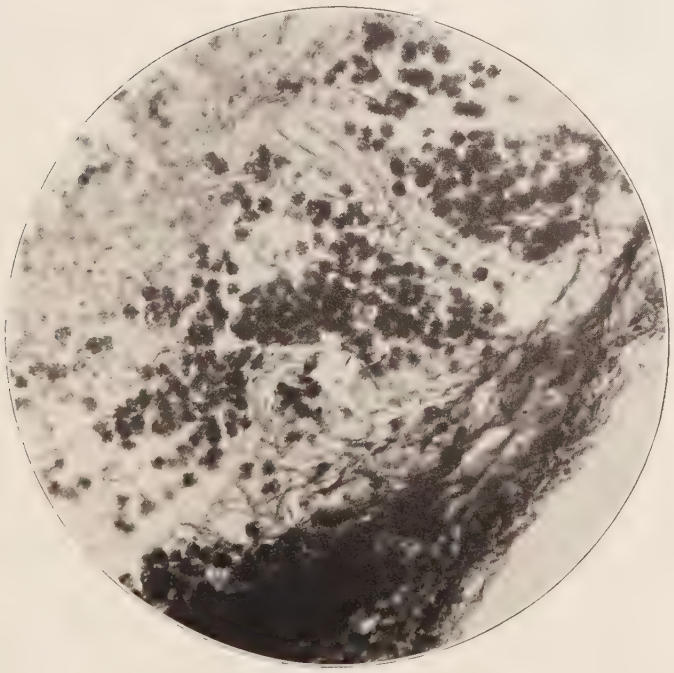

Figure 2

PLATE I

EXPLANATION OF PLATE I

Fig. 1.—Eosinophiles around air tube in lung, from colt II (Photomicrograph by A. Savage).

Fig. 2.—Eosinophiles grouped under covering of lung. Colt II (Photomicrograph by A. Savage).

SOME MARINE FISH TREMATODES OF MAINE*

HAROLD W. MANTER

During the summer of 1924 a study of marine fish parasites was made at the Mount Desert Island (Me.) Biological Laboratory. The present paper represents a preliminary report on the trematode group. Three new species of trematodes are described. Only brief consideration is here given to species already known. A later paper will deal more completely with these and related forms. The writer is indebted to Dr. H. B. Ward under whose direction the studies were undertaken. Gratitude is also expressed to Prof. Ulric Dahlgren for many courtesies received at the Mount Desert Island Biological Laboratory.

1. *Podocotyle atomon* (Rud. 1802)

From intestine, *Pholis gunnellus* (Butterfish), *Anarrhichas lupus* (Wolf-fish).

The forms described by Stafford (1904) and Cooper (1915) as *Sinistroporus simplex* can doubtless be referred to either *P. atomon* or *P. olssoni*. The chief distinctions between the two species are the longer esophagus, smaller testes, and continuous vitellaria of *P. atomon*. One specimen of this species was taken from each of two butterfish of ten examined.

2. *Podocotyle olssoni* Odhner 1905

From intestine, *Urophycis tenuis* (Hake), *Myxocephalus groenlandicus* ? (Sculpin) *Gadus callarias* (Cod).

These forms are apparently identical with the *Dist. simplex* of Linton (1898: 525).

3. *Stephanochasmus baccatus* Nicoll 1907

From intestine, *Hippoglossus hippoglossus* (Halibut).

This trematode agrees very well with the description of Nicoll (1913 a).

4. *Lepidapedon rachion* (Cobb.) 1858

From intestine, *Melanogrammus aeglifinus* (Haddock).

These trematodes are found only in very small numbers (one to three) in a single host. Odhner's (1905) extensive description of *Lepidora rachiaea* furnishes complete morphological data on the species.

5. *Lepidapedon elongatum* (Lebour 1908)

From intestine, *Urophycis tenuis* (Hake).

Five specimens were collected from a single host. They differ from Miss Lebour's (1908) description in not being more elongate than *L.*

* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 265.

rachion. An average sized specimen measured 2.4 by 0.54 mm. In other respects they resemble *L. elongatum.*

6. *Homalometron pallidum* Staff. 1904 (Figs. 1 and 2)

From intestine, *Fundulus heteroclitus.*

Stafford (1904) bases this genus on the form described as *Dist.* sp. by Linton (1901:422). Looss (1907:613-14) points out that the trematode seems to agree with the genus Lepocreadium of Stossich (1903). The most important features of this species are found in the male reproductive system. In Lepocreadium a prominent cirrus sac is present. It encloses the prostate gland and an anterior region of the seminal vesicle which is thus divided into two parts. In the present form from Fundulus, however, the cirrus sac is entirely absent and the globular seminal vesicle opens directly into the pars prostatica the glandular cells of which lie free in the parenchyma at about the level of the ventral sucker (Fig. 2). The genital pore is median.

7. *Steganoderma formosum* Staff. 1904 (Fig. 8)

From pyloric caeca, *Hippoglossus hippoglossus* (Halibut).

Six specimens were obtained from the pyloric caeca of a single halibut. The genus seems to resemble Lecithostaphylus Odhner, differing in its very small pharynx, long esophagus, elongate cirrus, well developed excretory vesicle, and Laurer's canal with pore. An outline of the genus based on Stafford's description with some additions follows:

Body somewhat elongate, regular in outline, flattened, both ends rounded, anterior end slightly broader. Scale-like spines cover body to near the posterior end. Suckers about equal in size, ventral sucker a little more than ⅓ from anterior end. Very small pharynx, long esophagus, caeca extending slightly more than half the body length. Ovary median or to one side, just posterior to the ventral sucker. Testes side by side at ends of caeca. Uterus between testes and filling posterior part of body. Cirrus sac elongate, almost straight, reaching posteriorly to and sometimes overlapping the ventral sucker, crossing left caecum between ventral sucker and the forking of the intestine. Genital opening ventral and to the left about half way between caecum and margin of the body. Vitellaria lateral, reaching from ventral sucker to the testes, composed of a few large follicles. Laurer's canal present. Seminal receptacle absent. Type species: *S. formosum* Staff.

8. *Otodistomum cestoides* (van Ben. 1870)

From stomach, *Raia laevis* (Barn-door skate)

Special consideration was given to this form and a careful study of its morphology, the hatching of its eggs, its miracidium, and growth

changes was made. Certain conclusions from this study were applied to various fresh-water Azygiidae. The results of this study will appear in a later paper.

9. *Hemiurus levinseni* Odhner 1905

From stomach, *Gadus callarias* (Cod), *Urophycis chuss* (Squirrel hake).

The *H. appendiculatus* of Stafford described as with "suckers of equal size" probably belongs to *H. levinseni.* Eighteen specimens were taken from the stomach of six squirrel hake. Six or eight specimens were collected from three cod.

10. *Brachyphallus crenatus* (Rud. 1802)

From stomach and intestine, *Osmerus mordax* (Smelt), *Pollachias virens* (Pollack), *Clupea harengris* (Herring).

Lander (1904) has described this form in detail. The present material shows distinctly lobed vitellaria which are not noticeably longer than wide. The assignment of the American form to a new species by Looss (1907 a) on the basis of shape of vitellaria was not supported by study of 32 specimens which seem to agree fully with the European *B. crenatus.*

11. *Lecithaster gibbosus* (Rud. 1802)

From intestine, *Myocephalus octodecimspinosus?* (Sculpin).

The two species *Lec. confusus* and *Lec. gibbosus* are closely related. The present specimen was assigned to the latter species because of the thickness of the ovarian lobes, the length of the vitelline lobes, and because the seminal vesicle does not extend posterior to the ventral sucker.

12. *Aponurus sphaerolecithus* n. sp. (Figs. 9-13)

From stomach, *Urophycis tenuis* (Hake).

Two specimens of this form were obtained from the stomach of a single host. Both specimens were mature and measured 1.47 by 0.29 mm. and 1.1 by 0.245 mm. Posterior to the ventral sucker the body is cylindrical. It is broadly rounded at the posterior end. The cuticula is smooth and there is no tail appendage. The sucker proportion is almost exactly 1 : 2. Pre-pharynx lacking, pharynx globular, esophagus short, wide caeca reaching to posterior end of body. The unpaired excretory vesicle branches between the ovary and testes, the two lateral branches uniting dorsal to the pharynx.

The genital pore is ventral, medium, at about mid-pharynx level. A muscular sinus sac, surrounding the genital sinus, extends dorsally and posteriorly from the pore. It reaches about half-way to the ventral sucker. The genital sinus coils slightly within the sac (Fig. 9). The testes are located a short distance posterior to the ventral sucker. In

both specimens the right testis was slightly anterior to the left. These organs are relatively smaller than in *A. laguncula,* the other species in the genus. The seminal vesicle is large and simple sac like in form. It extends posteriorly to near the middle of the ventral sucker. The duct of the pars prostatica leads from the ventral surface of the seminal vesicle near its anterior end, bends directly dorsally over the anterior end of the seminal vesicle, reaches to the dorsal body wall, and then bends ventrally to unite immediately with the uterus to form the genital sinus. The two ducts unite just within the posterior tip of the sinus sac. The prostate gland is free, S-shaped in lateral view, and its total length is just about equal to the length of the sinus sac.

The globular ovary is located posterior to the testes and slightly to the right. The seminal receptacle is about one-half the size of the ovary and located anterior and slightly dorsal to it. Laurer's canal is absent. The vitellaria consist of seven separate follicles posterior to the ovary. These follicles are in two groups, one of four, and one of three follicles. The largest follicles are about the size of the ovary. Cross-sections through the larger specimen give no indication that the follicles are united at any point. In spite of Looss's question, this character seems to be a distinct one, although more material will be necessary to settle the point finally. The eggs are very large and this character forms a conspicuous difference between the two species of Aponurus. Looss gives 27 by 76μ for the eggs of *A. laguncula.* Eggs in the present species measure 56 to 65 by 26μ.

General measurements on two specimens are as follows:

Length	1.47 mm.	1.1 mm.
Width	0.296	0.245
Oral sucker	0.137	0.1
Ventral sucker	0.264	0.19
Ant. end to post. edge ventral sucker	0.617	0.43
Pharynx	63 by 63 μ	57 by 57 μ
Diameter, testis (anterior)	68 μ	57 μ
Diameter, testis (posterior)	79 μ	68 μ
Diameter, ovary	91 μ	85 μ
Diameter, vitellaria	85–91 μ	62–72 μ
Eggs	58-62 by 26 μ	56-65 by 26μ
Seminal vesicle	0.188 by 0.1 mm.	0.143 by 0.088 mm.
Sinus sac (length)	.....	0.2
Prostate gland (length)	.....	0.19

The genus Aponurus (Looss, 1907) bears close resemblance to Lecithaster and Lecithophyllum. The present form is placed in the genus Aponurus because it agrees with that genus in the following points: the genital sinus extends only half-way to the ventral sucker; the pars prostatica is about equal in length with the sinus sac; and the vitellaria are in seven separate parts. It differs from *A. laguncula* in having large eggs (as found in Lecithophyllum) and in its seminal vesicle which is simple-sac like in shape and extends slightly more posteriorly (to about the middle of the ventral sucker).

13. *Genolinea laticauda* n. g., n. sp. (Figs. 3-5).

From stomach, *Hippoglossus hippoglossus* (Halibut).

Small, to medium-sized forms, with flattened body, tapering slightly and broadly pointed anteriorly, but broadly rounded posteriorly. Body almost uniformly wide. Cuticula smooth. Tail appendage lacking. Oral sucker embedded in body, overlapped dorsally by fleshy lip. Ventral sucker about 1½ times the size of oral sucker, located about at end of first body third. No pre-pharynx, pharynx broad, esophagus very short, caeca wide, extending to posterior tip of body. Excretory system as in other Hemiuridae, branches uniting dorsal to pharynx. Genital pore ventral, median, at about the level of the forking of the intestine. Testes globular, obliquely behind one another some distance behind ventral sucker. Ovary large, globular, behind testes. Vitellaria behind one another posterior to ovary. Uterus sends two lateral coils posterior to vitellaria to near body tip. Between the ovary and ventral sucker the uterus is in large, transverse coils. Genital sinus short, sinus sac present, pars prostatica short, seminal vesicle much coiled, just anterior or slightly overlapping the ventral sucker. Eggs 28 to 31 by 12 to 15μ.

This form is most closely related to Derogenes Lühe and Genarches Lss. It differs from both in body shape which is not tapering at either end, and in position and proportional size of the ventral sucker, which is distinctly anterior to mid-body. The uterus in the present form also has a distinct course differing from both Derogenes and Genarches. The most marked difference between Genarches and Genolinea is the fact that in the former genus the two caeca of the intestine are continuous with each other posteriorly. From Derogenes, the new genus, in addition to points already mentioned, is distinct in possessing a very short prostate gland, much coiled seminal vesicle, and in a more linear arrangement of the reproductive organs.

Measurements on two specimens are as follows:

Length	1.87 mm.	1.32 mm.
Width	0.336	0.299
Oral sucker	0.136	0.125
Ventral sucker	0.239	0.199
Ant. end to post. edge ventral sucker	0.617	0.5
Pharynx	57 by 79 μ	57 by 74 μ
Testis (anterior)	0.136 mm.	0.1 mm.
Testis (posterior)	0.136	0.13
Ovary	0.165	0.15
Vitellarium (anterior)	0.114	0.12
Vitellarium (posterior)	0.142	0.12
Eggs	31 by 13-15 μ	28-31 by 12 μ

14. *Gonocerca phycidis* n. g., n. sp. (Figs. 6-7)

From gills, branchial cavity, and stomach, *Urophycis chuss* (Squirrel hake).

Body elongate, both ends bluntly rounded, cuticula smooth, body only slightly flattened, oval in x-section, tail appendage lacking. Ventral

sucker posterior to mid-body, almost twice as large as oral sucker, about as wide as body. Mouth opening sub-terminal, overlapped dorsally by lip-like projection of body, oral sucker embedded in body. No prepharynx, short esophagus, caeca reaching to posterior end of body. Excretory vesicle branches just posterior to the ovary, the branches uniting dorsal to the oral sucker near the anterior tip. Gonads crowded together posterior to the ventral sucker and filling most of body space in that region. Genital pore ventral, median, close behind mouth opening. Ovary median just behind ventral sucker, anterior to testes. Vitellaria unlobed, lateral and very slightly posterior to ovary. Ootype without membrane, dorsal and anterior to ovary. Laurer's canal present. Uterus entirely anterior to ovary. Eggs large. Testes large, just posterior to ovary, obliquely behind and in contact with each other. Seminal vesicle comma-shaped, pointed anteriorly, located at about the level of the pharynx. Prostate gland little developed, free, short, located ventral to the oral sucker just anterior to the seminal vesicle. Cirrus sac absent. Genital sinus short. No localized seminal receptacle. About 15 specimens were taken from the gills and branchial cavity of a single host. Two specimens were obtained from the stomach.

Gonocerca shows resemblance to Liocerca Lss. which is also a gill parasite and one of the few Hemiurids with testes posterior to the ovary. Hemipera Nicoll (1913) also shows this feature. A tabular comparison of the three genera follows:

	Liocerca	Gonocerca	Hemipera
Habitat	Gills	Gills	Stomach
Position of genital pore	Somewhat distant from oral sucker	Close to oral sucker	Somewhat distant from oral sucker
Position of ventral sucker	About mid-body	Posterior to mid-body	Posterior to mid-body
Testes	Behind one another	Behind one another	Lateral to each other
Cirrus sac	Inclosing only male duct	Absent	Inclosing prostate gland and sem. vesicle
Prostate gland	Free, elongate	Free, short	Inclosed
Seminal vesicle	Near ventral sucker	Near pharynx	Between suckers
Eggs	Non-filamented, numerous	Non-filamented numerous	Filamented, few

Form, shape, size, cuticula, excretory and digestive systems are similar in all three genera.

Meaurements on five specimens of *Gonocerca phycidis* are as follows:

Length	1.8 mm.	1.9 mm.	1.3 mm.	1.4 mm.	1.4 mm.
Width	0.48	0.37	0.29	0.37	0.4
Ant. end to post. edge ventral sucker	1:3	1.3	0.89	1.	1.
Oral sucker	0.26	0.22	0.18	0.2	0.22
Ventral sucker	0.43	0.37	0.29	0.33	0.35
Testes	0.22	0.19	0.17	0.23	0.23
Ovary	0.2	0.12	0.13	0.12	0.125
Vitellaria	0.15	0.1	0.1	0.114	0.114
Pharynx	114 by 85 μ	80 by 80 μ	74 by 85 μ	96 by 51 μ	85 by 79 μ
Eggs	....	....	46-50 by 20-26 μ	....	....

15. *Derogenes varicus* (Müller 1784)

From stomach, *Gadus callarias* (Cod), *Urophycis tenuis* (Hake), *Urophycis chuss* (Squirrel hake), *Anarrhichas lupus* (Wolf-fish), intestine, *Hippoglossus hippoglossus* (Halibut), *Myxocephalus octodecimspinosus* (Sculpin).

16. *Acanthocotyle verrilli* Goto 1899

From body surface, *Raia erinacea* (Bonnet skate).

A single specimen of this species was collected. The species is described by Goto (1899) from a single specimen sent him by Verrill who obtained it from the surface of a "skate" (from Cape Cod).

Monticelli (1904: 73-74) insists that the anterior "invaginations" of Goto are true suckers, and this conclusion is supported by study of the specimen at hand. Goto was also in error (as Monticelli suggests) in respect to the genital openings which are three in number as in other species of the genus. Monticelli also believes Goto wrong in regard to the metraterm opening to the right. In the present specimen there is no doubt, however, that this pore is on the right side. This condition may, of course, represent a case of amphitypy.

17. *Dactylocotyle minor* (Olss. 1868)

From gills, *Urophycis chuss* (Squirrel hake).

Three specimens were collected from the gills of a single fish, but the parasite is not common. It cannot be referred to *Dact. phycidis* because of its marked difference in size, shape, and number of hooks in the genital sucker. The live worms are of a gray color. There are 14 hooks in the genital sucker. A single nonfilamented egg in the vagina measured 159 by 17μ. There is a common genital opening.

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Explanation of Plate II

All drawings were made with the aid of a camera lucida, and the projected scale has the value of 0.1 mm.

Abbreviations: *cs,* cirrus sac; *dp,* duct of prostate gland; *e,* excretory system; *es,* esophagus; *gp,* genital pore; *gs,* genital sinus; *lc,* Laurer's canal; *o,* ovary; *ov,* ovum; *pr,* prostate gland; *sr,* seminal receptacle; *ss,* sinus sac; *sv,* seminal vesicle; *t,* testis; *u,* uterus; *v,* vagina; *vt,* vitellaria; *yd,* yolk duct.

Fig. 1.—*Homalometron pallidum.* Ventral view.

Fig. 2.—*H. pallidum.* Sagittal section through region of seminal vesicle.

Fig. 3.—*Genolinea laticauda.* Ventral view of anterior body region.

Fig. 4.—Ventral view of entire body of *G. laticauda.*

Fig. 5.—Same, of another specimen.

Fig. 6.—*Gonocerca phycidis.* Ventral view.

Fig. 7.—Ventral view of anterior body region of same.

Fig. 8.—*Steganoderma formosum.* Ventral view.

Fig. 9.—Lateral view of anterior body region of *Aponurus Sphaerolecithus.*

Fig. 10.—Ventral view of entire body of *A. Sphaerolecithus.*

Fig. 11.—Cross-section through body of *A. Sphaerolecithus* just posterior to sinus sac.

Fig. 12.—Ventro-lateral view of body of *A. Sphaerolecithus* in region of gonads.

Fig. 13.—Ventral view of same region.

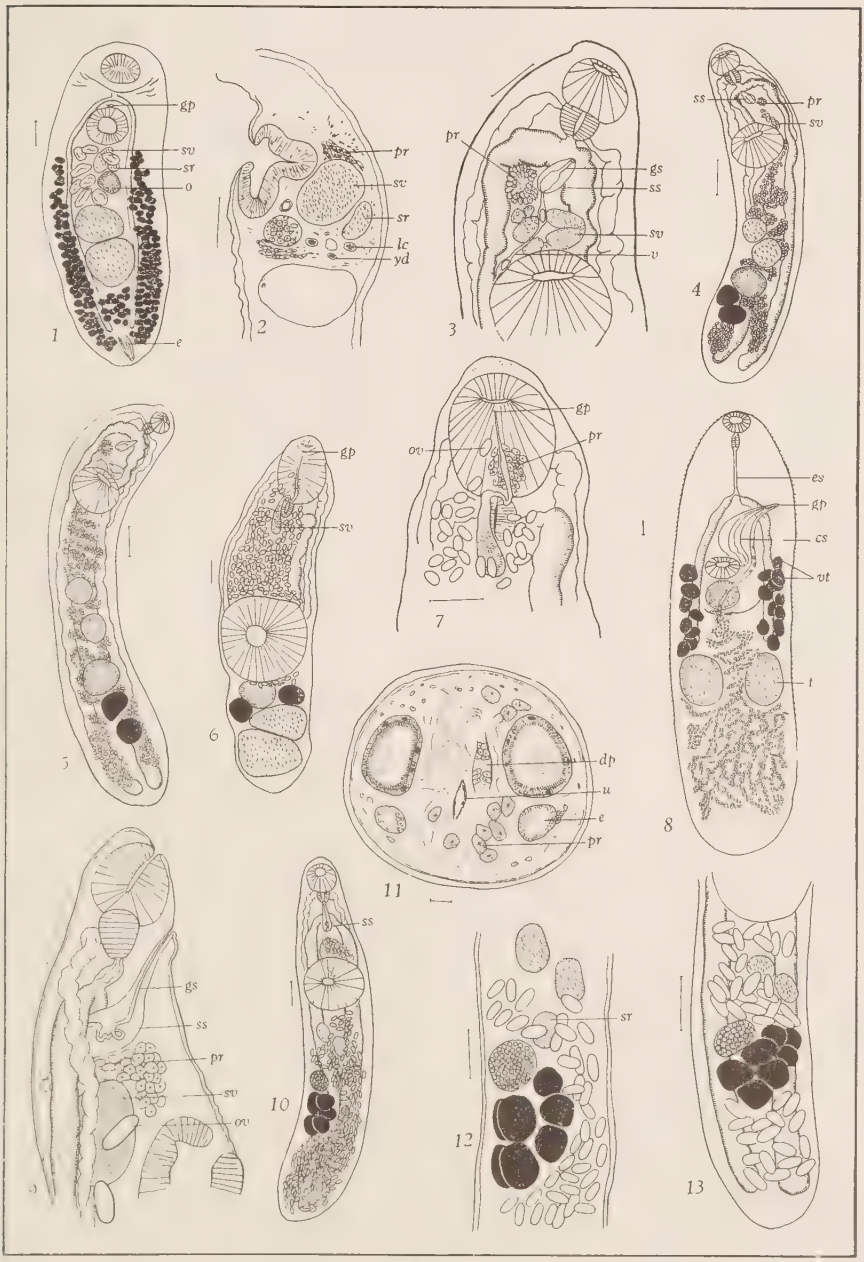
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PLATE II

HEPATICOLIASIS

A FREQUENT AND SOMETIMES FATAL VERMINOUS INFESTATION OF THE LIVERS OF RATS AND OTHER RODENTS *

FRED D. WEIDMAN

The whole subject of hepatic trichosomiasis is worthy of discussion, first because it calls attention to liver lesions of rodents which, according to Galli-Valerio might be confounded with those of plague (and, less importantly, with coccidiosis according to Bancroft), and secondly because any disease which will kill rats, rabbits and other noisome animals without also affecting important other ones deserves to be advertised, investigated and perhaps disseminated as a means of extermination. Thirdly, there is reason to believe that it has been responsible for a notable reduction in the population of the prairie dog enclosure at our Zoological Garden in spite of known additions through breeding.

That this disease, preeminently one of rats, is widespread might be expected; it has been frequently reported in Germany, France, Italy and Australia; and in the United States, from Philadelphia, Rhode Island, Washington, D. C., and San Francisco. Previously recorded hosts are the Spitz mouse (1), the Albino rat, wild rats (*Epimys norvegicus* and *Epimys alexandrinus*) and, questionably, the European hare (*Lepus europus*). Some records of the structure of the worm, its life history, etc., may be found in the articles by Bancroft (1893) and Hall (1916).

CLINICAL OBSERVATIONS

Our first experience in the Garden was with an adult prairie dog (*Cynomys ludovicianus*) which died on July 3, 1915. It was said to have been in the Garden ten weeks, a statement which cannot be made beyond cavil, since the same enclosure contained goodly numbers of specimens of similar size and appearance. No symptoms had been noticed before death, the animal being dead when picked up by the keeper. No rats had been observed in the enclosure for many months, nor have they been seen since; but a squirrel was once seen coming from one of the burrows.

As seen at autopsy this prairie dog was a large specimen, well nourished, and showed no external abnormalities save distention of the abdomen. On opening the body a definite excess of ascitic fluid escaped and an enormously enlarged liver came into view. The pericardial sac

* From the Laboratory of Comparative Pathology of the Philadelphia Zoological Garden.

also contained a distinct excess of clear watery fluid. The lungs showed congestion and edema; the heart, pancreas, kidneys and intestines appeared normal. Unfortunately the whole body was not weighed, which prevented comparison of the several organs with the whole body weight.

The liver was the most conspicuous feature noted at autopsy. It measured 11 by 7 by 2.5 cm., weighed 90 gms., maintained its shape perfectly, had sharp edges, was pale clay yellow in color and was as hard as any liver, human or subhuman, that the writer has ever palpated. Its surface showed no adhesions, and while finely granular, was generally even. A gross diagnosis of fatty cirrhosis was given. The gall bladder was of normal size (25 by 8 by 8 mm.), its walls were of proper thickness and the bile was normal in all respects.

Two other prairie dogs subsequently came to autopsy from the exhibition enclosure with similarly affected livers, and by killing a newly purchased normal animal, it was possible to estimate the degree of liver enlargement. This is brought out in the following table:

Increase in Weight of Liver on Basis of Body Weight

	Normal	Animal No. 1 PZG3605	Animal No. 2 PZG5071	Animal No. 3 PZG5080
Total body weight	1090	Not taken	759	421
Weight of liver in grams	33	90	93	66
Ratio of liver to body weight	1:33	..	1:8	1: 6.5
Increase in weight of liver	....	..	400% (about)	500% (about)

The normal animal and Nos. 1 and 2 were mature; they had attained approximately the same level of physical development, since their kidneys, the weights of which do not vary as widely in disease as the liver, weighed 6.4, 6.5 and 7.0 gms.—practically the same. The liver then was certainly twice or thrice the normal size, and probably four or five times—a degree rarely approached in any disease process.

The spleen was moderately enlarged. It was slightly increased in firmness. Its section surface was bright red and the trabeculae slightly increased in prominence. Other organs appeared normal to the naked eye.

The microscopical examination showed a normal kidney, pancreas and intestines.

The capsule and interstitial tissue of the liver were of normal weight, but the perilobular portions were distinguishable only with difficulty and the lobular outlines were vague on account of the marked disorganization produced by the presence of the parasitic ova. These, which at first sight suggested whipworm eggs, conformed precisely to Hall's illustrations of Hepaticola, and do not have the button of

Trichuris. In places a rich lymphocytic infiltrate was noticed in the remnants of the perilobular fibrous tissue, but on the whole the tissue reaction was scanty. No ova were found in the portal venules. Bile ducts were distinguishable with difficulty; they were small and had practically no lumina. No distended examples were found. Blood capillaries were congested and liver cords narrow as though from pressure.

Several crush preparations and sundry incisions of the liver substance were made in a search for the mature worms, but none was found. A solitary larval worm, however, was discovered. It measured 0.9 mm. long and 0.02 mm. in its widest part, and lacked a sheath.

The pathological diagnosis showed marked edema of lungs, ascites, hydropericardium, infestation of liver by parasitic ova, congestion of spleen and kidney, and sub-acute enteritis. Death in this animal, recalling the ascites and the hydropericardium seen grossly and the atrophy of the liver cells seen microscopically, was doubtless due to the liver lesion.

This parasitic finding at once raised several questions: First, how many of the prairie dogs in the collection were similarly affected? Since the natural route of egg escape would appear to be via the bile ducts and intestines, all of these tracts were examined, but with negative results. In addition, fifteen separate samples of feces from the prairie dog enclosure were examined, as well as a large composite specimen, but with no parasitic findings. It appears that the ova do not pass from the liver by way of the intestines. Diagnosis must rest at present, therefore, on laparotomy, which is practical only in experimental work; and the condition of the remainder of our animals must remain for the present undetermined. Transmission of the disease, ipso facto, can probably only be effected after the liver has decomposed for several months and thereafter been eaten. Prairie dogs are cannibalistic; they have been observed eating their dead fellows and also dead rats.

Second, the identity of the parasite. The morphology of the ovum, its location and the host (a rodent) at once suggested *Hepaticola hepatica* and led to a comparison of the disease in the two hosts. Autopsies on gray rats caught both in the Garden and at the Philadelphia General Hospital (a distance of some two or three miles from the Garden) showed frequent involvement of the liver by *H. hepatica* lesions as described by Galli-Valerio and Bancroft, but never of the grade seen in the prairie dog. Generally we found five or six nodules about the size of a mustard seed; or at most a patch 5 mm. in diameter might appear, generally far around on the right side of the liver and on its lower border. They were never found in very young rats. Bancroft, in Brisbane, Australia, found "almost every rat affected, even the very young."

Microscopically, the lesions of the rat did not always contain ova, particularly when they were of small size and in small numbers. These smaller examples consisted of fibrous tissue and probably represented foreign body tubercles which had developed perhaps among the remnants of a dead male. They were not likely to be dead females since the ova undergo calcification with age (Bancroft) and would be recognized as such. The larger lesions, which did not contain ova, differed from those of the prairie dog in the notable tissue reaction around them, consisting in places of central collections of leucocytes (which could be seen infiltrating a disintegrating worm) and a very heavy peripheral fibrous wall. The ova were often retained in a necrotic epithelial lined tube of some sort, giving the idea that the eggs had not passed from the worm's body but lay in their original positions in the necrotic oviduct (cf. *Syngamus trachealis* of birds). In the prairie dog's liver no such location was noted, the ova lying diffusely or at most in galleries of the liver substance and eliciting no tissue reaction whatever.

Ova from various rats and prairie dog sources are compared in the following table:

OVA OF *Hepaticola hepatica*

	Length	Width
A. Bancroft's figures	55 μ	30 μ
	55	35
B. Prairie Dog, crushed liver	55	31
	57	32
Histological sections	52	30
	52	29
C. Philadelphia General Hospital rats, crushed liver	52	29
	52	29
	52	28
	50	29
Histological sections of liver	55	27
	50	26
	48	28
Philadelphia Zool. Garden, rats. Histological sections of liver	56	32
	52	29
	52	30

The measurements, then, of the prairie dog ova were almost identical with those given for rats by Bancroft. Those of the Philadelphia General Hospital rats are smaller, and their proportions a little different. Thus, they appear longer in proportion to their breadth, and their asymmetry is more marked.

If diagnosis may be permitted in the absence of mature specimens, i. e., upon the egg characteristics alone, this prairie dog parasite is *Hepaticola hepatica*. The measurements and morphology of ova are close to those given by Bancroft, and practically identical with those of rats killed in the Garden. The similarity of the ova and the fact that the disease has been transmitted experimentally (see ff.) from rat to prairie dog and vice versa is strong although not conclusive evidence that the adult worms concerned are identical species.

TRANSMISSION EXPERIMENTS

Bancroft has already worked out the life history of this worm, transmitting the disease from gray to white rats and commenting on the long period which must elapse (at least five months) before the disease kills the experimental animal. According to him the fresh liver containing the ova, when fed to the animals will not reproduce the disease. It must incubate for three or four months in water before reaching the infestive stage. This I can confirm, as will appear from the following.

Of fifty-seven wild rats examined in the Zoological Garden, I found twenty-four with well developed Hepaticola infestment. Egg-bearing tissue from these was teased and kept both in tap water and in moist sand at 70 F. The former was found to be the better method. It was examined at intervals of a week or ten days. The granular centers of the ova divided into from 3 to 8 blastomeres in two months, and in five into a definite, fully motile larva. The larva, when warmed to 37 C. frequently moved within the egg, but never sufficiently to take up a new position.

I was struck by the notable variation in the precocity of development of different ova. Thus, whereas most were larvated, some had not developed at twelve months while others had become degenerate and distintegrated. The explanation of this need not be on the basis of "infertility." Recalling the unusual life history of this parasite, i. e., that it dies within the host and does not necessarily discharge its ova per vaginam, it must be realized that at the time of death the more recently formed ova may not have developed to a "viable" stage.

Beyond this intramural stage the larvae have never passed in our containers. The action of the rat's stomachs has been mimicked by gently shaking the fully developed larval forms in artificial gastric juice at 37 C.; and both with and without incubating over night. The larvae have never emerged. The scrapings of the gastric mucosa of rats, both gray and albino, have been similarly applied to larvated ova at 37 C. Pressure will cause the ovum to rupture and discharge its larva, but under these circumstances the larva is either motionless or will at most only sway gently for a few minutes and then cease motion for all time and distintegrate.

I have been successful, however, with feeding experiments. Seven prairie dogs were secured fresh from the dealer. In one which was killed and six which were subjected to laparotomy, the livers were found free of the disease. Ova from rats were incubated for ten months and 300 larvated specimens fed to a white rat and one of the prairie dogs. Following this the feces of both animals were examined daily for four days and again on the ninth day to ascertain whether larvae or ova may pass through the intestines at these early dates, as occurs in trichiniasis.

None was found. The white rat died of bronchopneumonia eleven weeks later with no Hepaticola lesions in its liver and therefore the prairie dog was refed a few months later with a second lot of ova which had been developing for fifteen months. At the same time another white rat was similarly fed as a control. This second rat died in six months and two filariform worms were found in its liver but no ova. Before the writer could determine their structural details they were lost, being eaten by cockroaches during a short absence from the laboratory room; but it is believed that they were adult male Hepaticola.

The prairie dog increased in weight during these months and appeared in perfect health. Twenty-one months after the first feeding, and eighteen months after the second, the animal was killed, autopsied, and found infested.

The animal was well developed and fat. No ascites or hydropericardium was present, such as were found in the "spontaneous" case. The liver was markedly enlarged, weighing 51.7 gm. and measuring 8 by 6 by 1.5 cm. It showed the same features grossly as the spontaneous case: large, hard and pale. On examining the surface closely, preferably with the loupe, fine, yellow, powdery granulations could be distinguished arranged in more or less curved lines. On teasing some of these in water they appeared as galleries in the liver tissue filled with ova. The latter showed early segmentation, but no larval forms. Microscopical sections showed features identical with those of the spontaneous case (3605).

As far as transmission of the disease from rat to prairie dog is concerned, then, I have succeeded; but I have failed to produce an invariably fatal disease. It was hoped that, if severe enough in the experimental prairie dog to kill it, one might consider it as a weapon against them in those sections of the United States where they are a nuisance. I still feel that the disease may be useful, but certain it is that one must await means for producing ova in large or practical quantities. It may be that, once rampant in the wild, it would be unnecessary to produce ova in the laboratory.

Perhaps the above findings explain why rats have been comparatively little affected by the disease. First, and most important, the ova require a long extracorporeal developmental time. Furthermore, most dead rats are promptly eaten by their companions, and it is only those that lie in inaccessible places for several months that furnish larvated ova to contaminate the rats' food, or be drunk. Secondly, in order to furnish a sufficiently extensive disease, the eggs must be ingested in large numbers at one time, a circumstance that the above natural history will not provide. Infestation promises best where the rat has occasional access to the abode of some infested animal that has been dead the necessary five

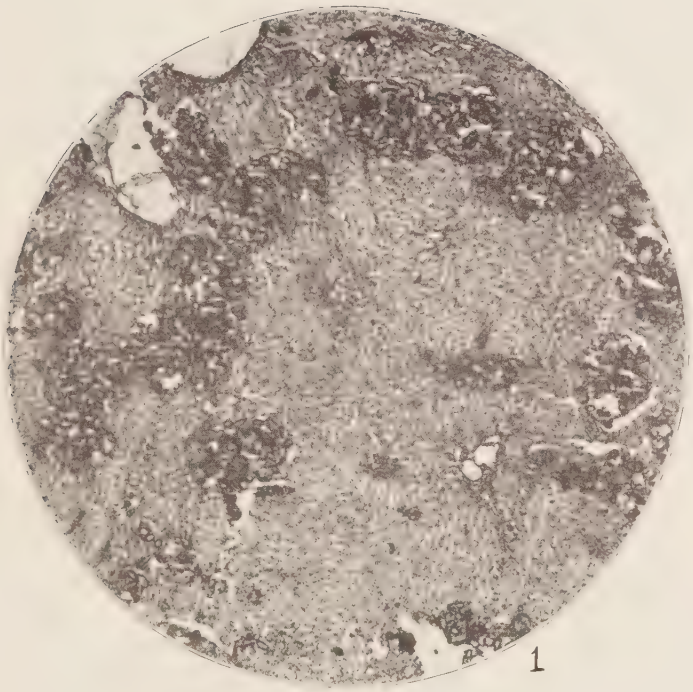
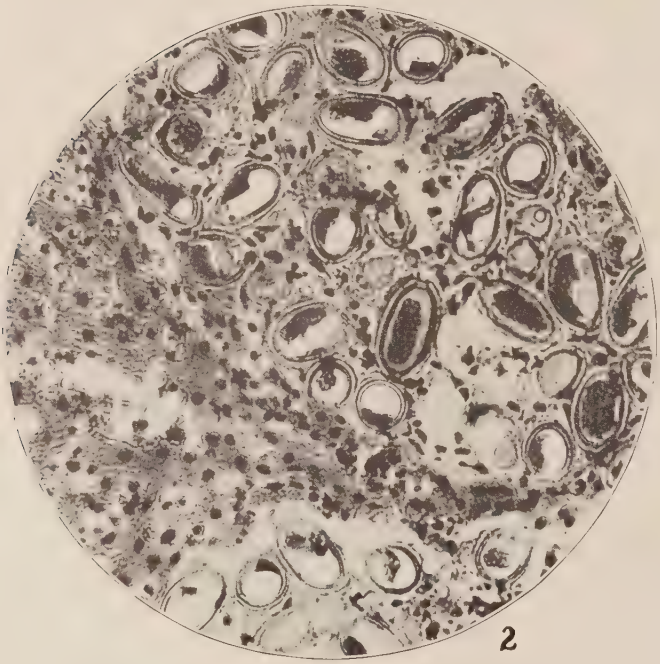


PLATE III

Explanation of Plate III

Fig. 1.—Prairie dog liver. Low power view, showing how the masses of ova (they appear in black) extend in tracts through the liver substance.



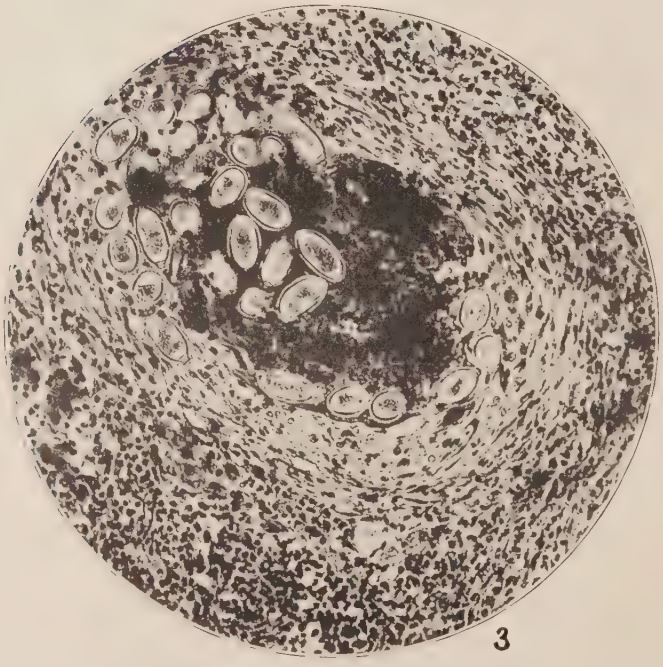


PLATE IV

EXPLANATION OF PLATE IV

Fig. 2.—Prairie dog liver. Disease experimentally induced by feeding ova from rat's liver. Very scant leucocytic reaction against the ova.

Fig. 3.—Rat liver. Intense reaction against the ova. The black material in the center of the lesions represents nuclear detritus and around this there is a dense fibrous wall, succeeded by a dense infiltrate of lymphocytes.

months and is now ripe for consumption, or that has disseminated the eggs in abundance over nearby food.

Altogether, five prairie dogs out of six autopsied have shown this disease during the ten years which have elapsed since the foregoing work was done. This makes a total of eight Hepaticola cases observed. During this time I have been awaiting the opportunity of teasing out an adult form, which was attempted in four cases, but without finding any. Even were they present, it would be a real feat to tease out the adult parasites in toto from such hard livers as those of the prairie dog, or at least in such shape as to permit specific determination. I feel that the best means of establishing the identity of rat and prairie dog parasites would be to feed prairie dogs' livers to white rats, and kill the latter at, say weekly intervals until the mature parasites were found in the liver. They would have to be teased or crushed out before the liver became hardened by the advancing disease.

In but three of the ten prairie dogs was there any ascites; but in all cases the livers were hard and yellow, and what is of particular diagnostic value, upon their finely granular surfaces delicate curved lines could be seen (preferably with the loupe), which betrayed the parasitic nature of the affection.

SUMMARY

Bancroft's work is confirmed as to (1) direct transmission of the parasite without the necessity of an intermediate host; (2) the long developmental period which must elapse after the death of the first host before the second becomes infested; and (3) that the ova do not pass from the body by the intestines.

As new findings are reported (1) the occurrence of probably the same worm in a new host, prairie dogs in the Philadelphia Zoological Garden. (This was probably acquired from the wild rats which are a pest in the Garden.) (2) Experimental transmission of wild rat infestation to prairie dog, and vice versa.

Material is filed at the Philadelphia Zoological Gardens (3605) and at the Army Medical School, Washington, D. C.

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ON DAVAINEA PROGLOTTINA (DAV.) AND ITS SYNONYMS*

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Since the first record and description of *Davainea proglottina* by Davaine in France, this tapeworm has been proved to be of worldwide distribution, occurring in nearly all countries of Europe, in North and South America, in Africa and Australia. In spite of this fact and in spite of the redescriptions of this worm by well known helminthologists, our knowledge of certain morphological features is still incomplete, so that it seems to be not without any reason that some workers considered certain closely allied forms to represent new varieties or even distinct species. Such forms as have been described up to the present time are the following: *D. proglottina* var. *dublanensis Kowalevsky* 1895, *D. varians* Sweet 1910, and *D. dubius* Meggitt 1916, all from the domestic fowl. The creation of these forms is based on certain peculiarities which are seemingly different from those exhibited by *D. proglottina* as described by Davaine, Blanchard and others. Fuhrmann (1919) in a recent paper, however, has shown that the specific value of these peculiarities is but limited when the different conditions of preservation and age of the specimens are taken into consideration. The discussion of this subject given by Fuhrmann (1919) as well as his completion of certain anatomical and morphological characters of these worms is based on preserved material and especially on specimens bearing no scoleces. It seems, therefore, that the question of the identity of the different, certainly closely allied worms mentioned above, is still unsettled.

The present writer had a favorable opportunity to carry out examinations on an almost inexhaustible supply of *D. proglottina,* the worms being present in certain flocks nearly in 80 per cent. of the cases. During the period of these examinations certain peculiarities of these worms were made out from which it became evident, or at least highly probable, that all the closely related worms described as distinct varieties or species are not more than synonyms of one common and widely spread tapeworm. The worm-material examined was collected from intestines of fowls which have been sent to the Bacteriology Department of the Michigan Agricultural College, either dead or mostly in living condi-

* Contribution from the Bacteriology Department of Michigan Agricultural College, East Lansing, with the permission of the Director of the Experiment Station, East Lansing, Michigan.

tion. They came from different parts of the State of Michigan; most of them, however, were received from the Poultry Department of the College, the intermediate hosts, slugs of at least two kinds, being of common occurrence in the poultry yard. For the purpose of morphological and anatomical examination only those tapeworms have been selected which were found in killed birds, postmortems on which had been performed almost without exception a few minutes after death. In cases of heavy infestation the worms could be easily detected with the naked eye; in such cases they cover by hundreds the surface of the intestinal mucosa. When the opened intestines are placed carefully in a dish containing tapwater or physiological salt solution, the bottom of the fluid becomes instantly covered with a large number of proglottids, usually of the same size, proving by this circumstance to be detached ripe proglottids of the worms, detachment having taken place probably in the living host's intestine. A slight shaking of the intestines in the fluid not only increases considerably the number of proglottids but also gives rise to the appearance of a number of entire worms. The size and shape of these latter or of the detached proglottids vary largely according to the nature of the fluid used. But they vary no doubt according to the age of the worms too. In tapwater the proglottids soon die and the ripe ones especially extend considerably, measuring 2 mm. or more in length and 0.8 to 1 mm. in width. In physiological salt solution they may live as long as 24 hours or more, the proglottids as well as the entire worms showing practically no change in size and shape. In examining birds which had been dead 3 to 4 hours or more I succeded only very rarely in obtaining entire worms with fully armed suckers and rostellum and never did so in birds which had been dead 24 hours or more.

These particulars, though not new to any parasitologist, seem to me of some importance in just this case, because in my opinion all discrepancies which exist in respect to the length of the worms, the length and shape of the proglottids, the armature of the rostellum and suckers, so far as *D. proglottina* and the related forms are concerned, are due mainly to factors resulting from the one-sided observation of an insufficiently large number of specimens.

DESCRIPTION OF THE WORMS EXAMINED

In trying to compare the forms described as varieties or distinct species with the specimens which in my opinion represent the true *D. proglottina,* I have considered it advisable to give a short description of the worms I had at my disposal.

Morphological features: The length varies a great deal, not only in specimens found in individual birds, for in most cases one and the

same birds harbors worms of different size, according to the number of proglottids and no doubt according to the age of the strobila. I wish to emphasize that the following measurements bear always upon mature worms. The smallest specimens consisting of four proglottids, the fourth one showing almost ripe eggs, measured 1.5 mm.; the largest worms, consisting of six, seldom seven proglottids, were 3.16 to 4 mm. in length. During my investigations I got the impression that the usual number of proglottids occurring in this tapeworm is 5 or in most cases 6. If the detachment of the ripe proglottids or the development of the eggs does not keep pace with the course of proliferation of new proglottids one can find 7 proglottids and under favorable conditions probably more. It seems that in this respect individual variations are likely to occur.

The scolex is almost spherical measuring in average 230μ (210-270μ) in length and 200μ in width. It bears a pillow-shaped (sometimes it may be more or less spherical, but doubtless in macerated specimens only) rostellum of 68 by 105μ in diameter, armed with two rows of small hammer-shaped hooks measuring in the anterior row 8μ in length; those of the posterior row are slightly smaller (7μ). Their number seems to be fairly constantly 94. The four suckers are 42μ in diameter and are armed on their anterior border with 4 rows (rarely there are additional hooks present so as to imitate a 5th row) of minute hooks; toward the posterior border their number decreases, but they are larger in size. There is no neck. The first proglottis, and in most cases the second also, is much broader than long. The third and very frequently the fourth proglottis are quadrangular, while the fifth and all subsequent proglottids are longer, usually two or three times longer, than broad. The ripe proglottids may attain the size of 0.8 to 1.5 mm. in length and 0.5 to 0.8 mm. in width.

Anatomical features: The most important anatomical features are the development and status of the genital glands in the different proglottids; these have been used quite extensively in separating the various allied forms from the typical *D. proglottina*. The male system consists of 12 to 19 testes situated behind the female organs in the posterior half of the proglottis. They are usually present in three subsequent proglottids, i. e., either in the second to fourth, or in the third to fifth proglottid showing full development mostly in the middle one (third or fourth), whereas the last one (fourth or fifth) shows them to be in regression. They measure 60 to 85μ in diameter. The cirrus pouch and vas deferens is present either in mere beginning, in full development, or in regression, in almost all proglottids except the first. There are but traces of these organs visible in very ripe proglottids. The genital openings are as a rule regularly alternating; exceptionally, however, one

may find two successive openings on the same side of the strobila. During the examination of hundreds of worms I observed this irregularity twice. The female glands are usually present (at least as a "trace") in the third proglottis; they are fully developed in the fourth and in regression in the fifth. A uterus with distinct walls is of very short existence, the eggs being scattered very soon throughout the parenchyma and enclosed singly in capsules formed by parenchym tissue. The eggs measure 35 to 40μ, the oncospheres 25 to 30μ in diameter.

COMPARISON OF THE DIFFERENT D. PROGLOTTINA-LIKE FORMS

A comparative study of *D. proglottina* var. *dublanensis* Kow., *D. varians* Sweet and *D. dubius* Meggitt with *D. proglottina* Dav. has been made, as mentioned above, by Fuhrmann (1919). From this study he draws the conclusion that *D. proglottina* var. *dublanensis* Kow. and *D. varians* Sweet are apparently synonyms of *D. proglottina* (Dav.). In an addendum to his paper, however, he mentions the recent publication of Meggitt (1916) in which is described a new species, *D. dubius,* closely allied to *D. proglottina,* and concludes that "D'après ce travail, nous sommes tenté de croire que le nombre de 80 à 95 crochets indiqué pour *D. proglottina* type, est erroné et, dans ce cas, *D. dubius* ne serait autre chose qu'une *D. proglottina.* Si par contre, l'indication du nombre des crochets du rostellum est juste, il faudra admettre que *D. dubius* Meggitt 1916 est une autre espèce, qui serait alors synonyme de *D. varians* Sweet 1910."

In my present paper it is shown that the worms I had at my disposal, exhibit features which are without any doubt characteristic for *D. proglottina* (Dav.). As, however, certain peculiarities of specific significance may be found equally in all other forms described as varieties or species, it seems advisable to compare these forms more closely with the typical *D. proglottina.*

DAVAINEA PROGLOTTINA *var.* DUBLANENSIS Kow., 1895

According to Kowalevsky the total length of this worm is 4 mm.; the strobila consists of six proglottids. The rostellar hooks (number not given) are said to agree fairly well in shape with those described by Blanchard (1895). The hooks of the suckers, however, are, according to Kowalevsky, different in shape from those present in *D. proglottina* type. The two last proglottids (5 and 6) attain together a length of 2.2 mm. The testes are fully developed in the fourth and fifth segment. Genital openings alternate regularly (exceptionally two successive proglottids may show the opening on the same side) and situated near to the anterior border of the segment.

Of all of these peculiarities there is not a single one which would not be equally applicable to *D. proglottina* as described above. Even the shape of the hooks of the suckers agree fully with those found in my specimens. It is, however, to be noted that the hooks on the suckers may be of different type according to their location. The larger hooks situated on the posterior border show exactly the same shape as Kowalevsky's figure 26.

DAVAINEA VARIANS Sweet 1910

The total length of this worm is said to be 0.7 to 1.8 mm.; there are usually 4 to 6 proglottids present. The rostellar armature consists of 44 to 50 hooks and 4 to 5 rows of hooks are on the suckers. In regard to the sex-glands it is stated that two possibilities may be observed: the testes are either present even in the fifth and sixth proglottis or they disappear very soon; eggs are present already in the third segment. The female glands are developed in the third and fourth proglottis.

From this description one would get the impression that *D. varians* represents a good species. There are, however, certain features which indicate that *D. varians* is nothing else than a *D. proglottina*. Such are: the armature of the suckers and the development of the sex-glands. The small size of the strobila is a matter of contraction or it may also prove that the worms were not fully mature. In my material I sometimes observed strobilae consisting of 5 to 6 proglottids all of which were broader than long and consequently the entire worm did not measure more than 2 mm. As the conditions in respect to the sex-glands are in accordance with those present in my specimens, I am of the opinion that the number given for the rostellar hooks is too low. I found specimens even among living ones which had lost the hooks of the lower row almost completely showing then a continuous row of anterior hooks numbering quite naturally not more than 47 to 50. Moreover, I had specimens with apparently full rostellar armature when the number of hooks was approximately 65 to 70 as seen in lateral view. Two specimens, however, in which the rostellum was in front view showed without any doubt the number of the hooks to be 94. It is probable that slight variations in this respect may occur. From the above comparison it seems beyond question that *D. varians* falls into synonymy with *D. proglottina;* this opinion was expressed by Johnston in 1912.

DAVAINEA DUBIUS *Meggitt* 1916

The most important features of this species in attempting to separate it from *D. proglottina* are, according to Meggitt (1916), the larger number of proglottids (there are usually 7 but as many as 9 may occur) and the number of the rostellar hooks (50 to 60 in two

rows). All the other characters said in Table 1 of his paper to be different from those of *D. proglottina* are of little value as compared with the corresponding features of *D. proglottina* simply because the latter do not represent the actual conditions of a typical *D. proglottina*. No differences have been observed between *D. dubius* and my worms so far as the anatomical structure is concerned.

In respect to the two first characters it seems they represent conditions which in a normal *D. proglottina* are sometimes rare and sometimes absent. In the description of the worms examined by me I mentioned that I found as many as 7 proglottids, though usually there were not more than 5 or 6, the latter number being the more frequent. Since, however, among the worms from certain birds I found frequently such with 5 proglottids, the fifth one containing fully ripe eggs and on the other hand in other birds I found specimens with 6 proglottids the sixth one being rather far from containing fully ripe eggs, I am of the opinion that under favorable circumstances there may be found strobilae of *D. proglottina* consisting of 7 and even more proglottids as well.

Finally in regard to the smaller number of the rostellar hooks it must be admitted that this difference is a considerable one though it seems to be of quite exceptional occurrence that a tapeworm of the family Davaineidae should exhibit in all morphological and anatomical details exactly the same structure as another so widely spread member of this family, except in respect to the number of the rostellar hooks.

Hence, in conclusion, while I believe that *D. dubius* does not represent a form differing specifically from *D. proglottina,* it may, however, be accepted as a local variety of this common tapeworm of fowl.

SUMMARY

A complementary description is given of *Davainea proglottina* with particular respect to the most important specific characters.

A comparative study of *D. proglottina* and the different closely allied worms shows that *D. proglottina* var. *dublanensis* Kow., *D. varians* Sweet, and probably *D. dubius* Meggitt also are only synonyms of *D. proglottina* (Dav.).

The writer is much indebted to Dean Dr. Ward Giltner, head of this department, for the opportunities given to him for work in this department. Cordial thanks are due to Dr. H. J. Stafseth and Dr. W. L. Chandler of the same department for their valuable support during the examinations.

NOTE.—Since the completion of the present paper the writer received a paper by C. R. Lopez Neyra (1920) in which the author is of the opinion that *D. proglottina* var. *dublanensis* Kow., *D. varians* Sweet, and *D. dubius* Meggitt are synonyms of *D. proglottina* (Dav.). Furthermore the present writer had the

opportunity of examining toto mounts of *D. dubius* kindly sent to him by Professor Meggitt. These specimens agree in general with *D. proglottina.* So that in conclusion *D. dubius,* like the two other forms mentioned above, without any doubt is a synonym of *D. proglottina* (Dav.).

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Explanation of Plate V

All figures represent *Davainea proglottina* (Dav.). Figs. 1 to 4. Young worms showing two, three, four and five proglottids respectively. Fig. 5, young mature worm showing six proglottids the last one being not fully ripe. Fig. 6. Ripe proglottis.

PLATE V

NOTES ON THE EFFECT OF BURIAL ON INFECTIVE HOOKWORM LARVAE *

W. W. Cort

In connection with expeditions to the West Indies for the study of hookworm disease, F. K. Payne (1922, 1923 and 1924) carried on experiments on the vertical migration of infective hookworm larvae. In the course of these experiments the startling discovery was made that larvae buried in loose sandy soil, the surface of which was subjected to alternate wetting and drying from violent tropical showers, could migrate to the surface from depths up to 36 inches. The maximum speed of migration was noted in larvae buried at a depth of 10 inches, which reached the surface in two days. The percentage of the larvae recovered from the surface decreased progressively with the increasing depth of burial. For example 62 per cent. of the larvae buried at a depth of 4 inches were recovered from the surface and only 3.6 per cent. of those buried to a depth of 18 inches. It was found that when the burial was in clay soil, the vertical migration was much less, in some types of soil being entirely prevented. In experiments in which the moisture content of the soil was kept uniform, few larvae were found to migrate upward more than two inches, and in saturated soil there was found to be a high mortality and almost no vertical migration. The suggestion was made that the larvae were oriented in their locomotion by movements of the soil water and that such movements played an important part in stimulating migration. Therefore, conditions that would produce movements in the soil water would be favorable to vertical migration. This work is not only a very significant addition to our knowledge of certain phases of the behavior of hookworm larvae, but also has far-reaching practical application in hookworm control work, since it defines the dangers involved in the burial of material containing viable hookworm eggs or larvae.

With Payne's work as a basis, in the summer of 1924, a series of experiments on the burial of infective hookworm larvae was carried out in Soochow, China, to test their ability to migrate in the clay-loam soil of the region and to get some idea of the extent to which buried larvae would be killed by the action of the sun's rays. The press of other work made it impossible to carry out as large a series of experiments as was

* This paper is a joint contribution from the Department of Pathology of the Peking Union Medical College and the Department of Medical Zoology of the Johns Hopkins School of Hygiene and Public Health. It forms a part of the researches of the China Hookworm Commission, the expenses of which were paid by the International Health Board of the Rockefeller Foundation.

at first planned. However, I am presenting the results obtained because some of the findings are suggestive and indicate lines along which further experiments are needed.

In carrying out my series of experiments, some 5-gallon oil tins were cut in half and buried in an exposed place. On the surface of the soil at the bottom of each were deposited 100,000 to 125,000 infective hookworm larvae, consisting of a mixture of *Ancylostoma duodenale* and *Necator americanus*. The surface at the bottom of the tins on which the larvae were placed was a hard packed clay. The larvae used were young and vigorous, having been isolated a short time before from cultures in the laboratory. The cans were then filled to a depth of 8 inches with moist soil which was allowed to settle naturally without being packed down. There was a distance of about 1½ inches between the level of the dirt in the cans and their exposed edges to prevent larvae from escaping. There was no rain during the course of the experiment, which lasted from July 24 to August 9, and the daily range of temperature for this period was from about 80 to 90 F.

These units were subjected to four different conditions. Four of them were left entirely exposed to the sun's rays, and the other four were kept constantly shaded by being covered with boards which also very greatly reduced evaporation. To two of the exposed cans and two of the covered ones enough water was added once each day to thoroughly soak the surface. The other four received no additions of water whatever. The soil used in these experiments was a clay loam. This soil was found to be somewhat unfavorable for the isolating of larvae with the Baermann apparatus, since in a considerable series of controls containing known numbers of larvae only between 50 and 60 per cent. of the larvae put in were recovered. These control isolations will be discussed in detail in another connection. For the examinations with the isolation apparatus of the surface of the units the upper 1 inch was carefully removed. After every second examination soil of as nearly as possible the same moisture content as that removed was added to bring the level up to 8 inches. It can be seen, therefore, that the larvae obtained from each surface examination had migrated vertically at least 6 inches.

The larvae were placed at the bottom of the tins on July 24 and the surface soil was examined at intervals up to August 9 (Table 1). On this date the total soil in each can was examined, layers a little over an inch in thickness being taken out separately (Table 2). I will attempt first by considering the findings from each series to give a picture of what probably happened under each type of condition. Although somewhat over a hundred thousand larvae counted by the dilution method were placed in each can it should be kept in mind that three factors in

the technic of the experiments would reduce the number which could be recovered. The first was that the isolations in the type of soil used were only a little over 50 per cent. effective, which would mean that in any given examination the larvae recovered would be only a little over half of those actually present. The second point was that in any miscellaneous batch of larvae a certain percentage will be either already dead or so weakened that they will soon die; and the third was that in handling the soil in such an experiment some larvae would undoubtedly be lost. I have no measure of the second two factors but they probably acted to a much less extent in reducing the numbers of larvae isolated than

TABLE 1.—*Numbers of Infective Hookworm Larvae Isolated from Surface Examinations of Burial Units*

Description of Units		Larvae Isolated from Surface					Total Recovered from Surface
		July 26	July 28	July 30	Aug. 4	Aug. 9	
A. Units not covered; no water added	I	0	0	0	0	0	0
	II	0	0	0	0	0	0
B. Units not covered; water added daily	I	63	1100	360	280	20	1823
	II	1186	900	360	280	0	2708
C. Units covered; no water added	I	0	20	0	0	0	20
	II	0	0	0	0	0	0
D. Units covered; water added daily	I	287	1820	780	1160	1040	5087
	II	1399	940	700	2260	1900	7199

TABLE 2.—*Numbers of Larvae Recovered at Different Depths at Conclusion Burial Experiment, August 9*

Description of Units		Larvae Isolated at Different Depths						Total Recovered Aug. 9
		1st, 1⅓′	2d, 1⅓′	3d, 1⅓′	4th, 1⅓′	5th, 1⅓′	6th, 1⅓′	
A. Units not covered; no water added	I	0	0	0	0	0	980	980
	II	0	0	0	0	300	2340	2640
B. Units not covered; water added daily	I	20	20	60	40	120	20	280
	II	0	60	20	0	20	120	220
C. Units covered; no water added	I	0	0	220	4080	19,920	5920	30,140
	II	0	0	20	4940	9700	5700	20,360
D. Units covered; water added daily	I	1040	300	120	60	220	*..	1740
	II	1900	200	180	140	820	540	3780

* The dirt on this can down to the solid substratum was exhausted in the first five samplings.

the first since the larvae used gave the appearance of being young, healthy and vigorous and the soil was all carefully handled by the writer himself. Since it is not possible to allow accurately for these factors, the percentage of larvae recovered will be considered in relation to the total number placed in each unit. The best idea, however, of the numbers which were killed under any given set of conditions will be gained by a comparison with the largest numbers isolated from any unit. It is also significant in considering the validity of the numbers isolated that the two units subjected to each type of condition checked quite well.

I will consider first the units *which were covered* and *to which no water was added*. It will be seen that under these conditions almost no larvae were recovered from the surface examinations (Table 1, C) and that when all the soil in the cans was examined on August 9 no larvae were found in the upper third and the great bulk were isolated from the deepest third. About 25 per cent. of all the larvae placed in these cans were isolated (Table 2, C) which indicates, when one considers the small percentage recovered in the other experiments and the three factors given above that reduced the possibilities of recovery, that the conditions were quite favorable for the persistence of the larvae. The surface of the soil in this experiment dried very slowly and most of the soil remained moist throughout the course of the examinations. Evidently there was little stimulus to these larvae to migrate vertically, but the fact that quite a large number had worked their way up from three to four inches suggests that had the experiment been continued longer they might have come up to the surface.

In the units *which were left uncovered* and *to which no water was* added there were no larvae isolated from the surface (Table 1, A) and the final examination (Table 2, A) showed a very great reduction since in one can less than 1 per cent. of the total number of larvae was recovered and in the other, between 2 and 3 per cent. The surface of the soil in these two units became baked dry within twenty-four hours after the beginning of the experiment, and by August 9 the dry zone had extended more than half way down the depth of the can. While the figures seem to show that there was little vertical migration in these units, a consideration of the conditions indicates that such a conclusion need not necessarily be drawn, since the larvae may have migrated upward and have been killed by the descending dry zone. That this is what actually happened seems very probable, since there was such a very great reduction in the numbers of larvae in these units and in the zones from which the few remaining larvae were recovered the soil was still moist at the conclusion of the experiment.

In the two *uncovered units* to which *water was added daily* the great bulk of the larvae were recovered from near the surface (Table 1, B), the largest numbers being found in the first two examinations. Only a comparatively few scattered larvae were found when the soil was completely examined on August 9. The total number of larvae isolated both from the surface and at the final examination was less than 3 per cent. of those put in. It seems probable that the important factor in this case in the rapid dying off of the larvae was the exposure of the surface to rapid drying of the sun's rays between waterings. Augustine (1923 : 427-428) has shown that in the alternate wetting and drying of a surface, large numbers of the larvae are caught and die from desiccation.

It would seem probable then that in these units the larvae were stimulated to rapid vertical migration, and their numbers were greatly reduced by the repeated drying out of the surface.

In the two units *which were covered* and *to which water was added daily,* there was also a large recovery of larvae from the surface (Table 1, D) indicating a rapid and extensive vertical migration. The fact that considerably less than 10 per cent. of the larvae put into these units were isolated both from the surface and in the final examination indicates a considerable mortality or loss of larvae. Drying was evidently not a factor here, since the soil in these units remained constantly quite moist. It is possible that in these two units larvae might have been able to migrate up the sides of the tin on to the covering board and in that way have been lost. Although the edge of the tin was about 1.5 inches above the soil, it seems possible that the saturated atmosphere in the top of this covered can produced by the evaporation from the constantly wet surface of the soil might have produced a sufficient water film on the insides of the tin for the migration of the larvae. I know of no other way to explain the great reduction of larvae in this experiment. In none of the other experiments could larvae have been lost in this way since in them, the inside surface of the tin above the surface of the soil was constantly dry.

Certain interesting conclusions are suggested by the results of these experiments. In the first place it is shown that in a period of a little over two weeks there was a great reduction in numbers of larvae which had been buried under 8 inches of clay loam soil, the greatest reduction being in the units *which were exposed.* Rapid and extensive vertical migration took place in the two sets to *which water was added* as shown by the larvae recovered from the surface and also probably in the *exposed units to which no water was added,* although, no larvae were isolated in this case from near the surface. In the *covered units to which no water was added* there was little evidence of vertical migration during the time the experiments were carried on. These findings support Payne's opinion that movements in the soil water are of importance in stimulating vertical migration.

This preliminary series of experiments suggest the possibility of testing the factors involved in a more extensive series in which a sufficient number of units would be used so that not only could the experiment be carried over a longer period, but that also at each examination the total soil in a can could be examined to follow the larvae from day to day both in their vertical migrations and their dying off. It seems to me that such a series would give us a rather complete series of pictures where a limited series such as that described above gives only glimpses of what is happening.

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AVERAGE EGG COUNT PER GRAM PER FEMALE HOOKWORM IN CEYLON *

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In a recent test of treatment methods, worm counts following trial and test treatments were made on fifty-two persons, residents of the Bogambara Jail, Kandy, Ceylon. The fifty-two persons were divided into four groups. Two of these groups received treatments of 2.4 c c. of carbon tetrachlorid given in a purgative solution of magnesium sulphate and two hours before such a solution, while the other two groups received 1.8 c c. of carbon tetrachlorid mixed with 0.6 c c. oil of chenopodium given with, and two hours before, a solution of salts. The test treatment consisted of 2 c c. of oil of chenopodium followed in one hour by a purgative dose of magnesium sulphate. Worm counts were made for the two days following each treatment. All persons treated were adult males, with ages ranging from 18 to 73, and all received the full dosage stated.

It was probable that the two treatments given did not remove as high a proportion of the worms of the persons treated as is usually removed when Darling's full test treatment is given and that the worms recovered did not represent the total worm content of the group. It seems likely, however, from previous experience that the two treatments did remove upward of 96 per cent. of all the worms present and that the error in results from this source was a small one. The worm counts were made carefully, but it is impossible to estimate the error in worm counts due to loss of worms in stool examination. This is an error inherent in the worm-counting method and is probably present to a certain extent in all worm counts reported.

Previous to treatment, one egg count by Stoll's method (Stoll, 1923 a, b, 1924) was made on one fecal specimen, collected in quarter-ounce tins, from each person treated. In the routine field use of Stoll's method, it is seldom possible to make more than one egg count per person, so this special group was included in this routine. If conclusions as to worm counts are to be based on field methods, tests of these methods should follow field procedure as far as possible.

All of the stools on which egg counts were made were classified under the Ceylon classification of soft. It is difficult accurately to classify stools from written descriptions, but it seems probable that these soft stools would be classified by Stoll as mushy. Practically the

* The work here reported was conducted with the support, and under the auspices of the Government of Ceylon and the International Health Board of the Rockefeller Foundation.

universal diet of Ceylon is rice and curries of various descriptions, all of which contain considerable waste material. The resulting stools are mainly rather soft and it is seldom that undeniably formed stools are seen. Since such a large majority of the stools of Ceylon are of this character, field egg counts are made on soft stools only, formed and watery stools being discarded.

Table 1 gives the data obtained in regard to egg counts, female worm counts and their relation. There were 3,767 male hookworms recovered in a total of 8,348 worms for the fifty-two persons, an average of 160 worms per person. The male: female ratio is as 1 : 1.22, low for the males. All the worms recovered were of the species *Necator americanus*. Table 1 is divided into the four treatment groups; Group 1 received the combination treatment followed by salts in two hours; Group 2, carbon tetrachlorid followed by salts in two hours; Group 3, carbon tetrachlorid in salts, and Group 4, the combination in salts.

TABLE 1.—*Data on Average Egg Count Per Gram Per Female Hookworm from Fifty-Two Prisoners in Kandy, Ceylon*

	Group 1	Group 2	Group 3	Group 4	All Groups
Number of persons in group.....	15	14	10	13	52
Total eggs per gram of feces.....	31,400	33,700	13,100	39,200	117,400
Average eggs per gram per person.....	2,093	2,407	1,310	3,015	2,258
Total female hookworms recovered.....	1,722	1,404	301	1,154	4,581
Average females per person.....	115	100	30	89	88
Average eggs per gram per female worm.	18.2	24.0	43.6	33.9	25.6
Number of male hookworms recovered...	1,393	1,023	228	1,123	3,767
Total hookworms recovered.....	3,115	2,427	529	2,277	8,348
Average number hookworms per person	208	173	53	175	160

The average number for all groups of eggs per gram of feces per female hookworm recovered was 25.6. This figure is practically identical with Stoll's figure (1923 b) of 25 eggs per gram per female worm for mushy stools. As has been already stated, it seems probable that the soft feces of the Ceylon classification would come under Stoll's class of mushy stools.

In a later article Stoll suggested that all egg counts based on mushy stools should be multiplied by two to reduce them to a "basis of formed stools." If this is done in the present series, the total egg count per gram becomes 234,000. This divided by Stoll's factor of 44 gives the total number of female hookworms as 5,336, an average of 103 per person, while the actual average found was 88. These two figures are in the same degree of infection and the theoretical number obtained by Stoll's factor is highly satisfactory. It has already been pointed out that the treatments given in this series probably did not remove quite all the worms present.

The only way of estimating the total number of males and females from Stoll's factors is to multiply the number of female worms obtained

by two. That process would give the average number of worms per person in these four groups as 206, while the actual average recovered was 160.* This is an error of 46 worms per person, which would be considerable when accuracy is essential. In routine field work, however, and in the drawing up of plans for permanent control, this error would not assume much importance and would, furthermore, be in the right direction since it would lead to an overestimate of the infection present. Also in field work, where large groups of infection rates are made, it is probable that these two figures would fall in the same group of infection and receive the same control methods.

There were very considerable variations in the factors obtained in the four groups of this series for eggs per gram per female worm. The variations were much greater when this factor was obtained for each person in the group and the average taken for the factor for the group. When this latter method was adopted the factors were found to be as follows: Group 1, 34; group 2, 56; group 3, 85; group 4, 39; all groups, 51.

The factor obtained for the whole series by this method was 51, with individual factors ranging from three to four hundred. The factor 51 is practically double the 25 obtained by general averages and would give a corresponding underestimate of the number of worms per person.*

It seems apparent that one examination by the egg-counting method cannot be used for estimating the infection of individuals nor of small groups. Repeated examinations in these cases would probably make the method of value but the question as to size of group necessary for accuracy, when only one examination is made, remains to be answered. From the study of something over 5,000 egg counts, the writer is inclined to think that the smallest group possible for any degree of accuracy is in the neighborhood of fifty. This, however, is still problematical and requires much more work.

The factor obtained by a general average of all the fifty-two persons in this series seemed more nearly correct than the factors obtained in any other way. The egg-counting method is one for averages and not for individual use. In summarizing field reports of egg counts, it seems probable that the average egg count for the whole series should

* Provided a 1:1 ratio of males and females had been obtained in our worm counts, this "actual average recovered" would have been 176 instead of 160, which would mean a discrepancy of but 30 worms between the egg count estimate and the "actual average recovered."

* It should be pointed out that this difference has its origin in the great amount of variability found from case to case in the eggs per gram per female worm ratio. Stoll's Porto Rico figures were much more uniform and result in about the same factor figured either way.

be obtained first and the average worm count figured from this average. The use of factors to get worm counts of individual cases and the averaging of these individual worm counts would probably lead to much larger error in results.

It is evident, however, that in Stoll's egg-counting method, we have a means, sufficiently accurate, for the rapid estimation of degree of infection in large groups. Its routine field use, with the use of Stoll's factors of 44 and 25, will give a workable knowledge of the degrees of infection in Ceylon, a knowledge which can be depended on for the recommendation of control methods. The weakest point in the use of the method, at present, would seem to be the matter of a final factor to be used for converting egg counts into numbers of worms. The advantage, for government and public uses, of an infection index based on numbers of worms per person is obvious, although not axiomatic and the determination of a reliable factor is needed if this advantage is to be gained. The series here reported is too small to be of great value, but indicates that Stoll's factors are the ones to use in Ceylon. Whether these factors vary with changes in diet and other influences is a question which needs more study.

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NOTES ON THE SIPHONAPTERAN GENUS DORATOPSYLLA JORDAN AND ROTHSCHILD

TOGETHER WITH A DESCRIPTION OF A NEW GENUS AND SPECIES OF FLEAS

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The genus Doratopsylla was established in 1912 by Jordan and Rothschild for a species of Typhlopsylla, *T. dasycnemus* Rothschild. Later (1915) Rothschild referred to this genus five more species, including two new ones *D. curvata* and *D. cuspis,* and gave it the following diagnosis: "All of them (Doratopsylla species) are characterized, *inter alia,* by the labial palpus consisting of four segments, the genal comb containing four spines, the fifth segment of all the tarsi bearing four lateral pairs of plantar bristles and an additional pair (the true first) in between the first lateral pair, the antennal groove being closed, the frons rounded, the stigma of the eighth abdominal segment large, and the pygidium convex."

Rothschild also points out that the type of Cunha's genus Stenopsylla, *S. cruzi* Cunha, must be considered as a synonym of *Doratopsylla intermedia* (Wagner), and accordingly he suppressed the genus Stenopsylla. Recently the writer has had the opportunity of studying material representing four species of the genus Doratopsylla and offers the following notes.

THE GENUS DORATOPSYLLA JORDAN AND ROTHSCHILD

This genus can easily be divided into three groups of species which in two cases at least must represent natural divisions of very closely related components. Of the seven included species (counting the one described in this paper) five have three antepygidial bristles, the dorsal process of the male clasper represented by two lobes and the movable finger slender and not inflated posteriorly. The other two species, *D. antiquorum* Rothschild and the new one described in this paper, have but two antepygidial bristles (in addition to a minute hair), the dorsal process of the male clasper represented by a single lobe and the movable finger inflated posteriorly. The five species placed in this first division may again be divided into two groups, one including *D. intermedia* Wagner and *D. curvata* Rothschild, in which the anterior lobe of the dorsal process of the male clasper is very long and conspicuous and bears two large similar setae and the other group, including *D. dasy-*

cnemus Rothschild, *D. blarinae* Fox and *D. cuspis* Rothschild, in which the anterior lobe of the dorsal process of the male clasper is very low and greatly reduced but bears three large setae, one of which is flattened or of a different form from the others.

While suppressing the genus Stenopsylla Cunha, Rothschild (1915) states: "If at a later date the genus should require to be divided into several genera, the name Stenopsylla will be available for *intermedia* Wagn. (1901) = *cruzi* Cunha (1914)." As just stated, *intermedia* Wagner and *curvata* Rothschild have the same type of genital armature. If some character other than a sexual one also could be found for separating these two species from the remaining ones of *Doratopsylla* a justification for the recognition of Stenopsylla would be found. So far such an additional nonsexual character has not been found. Hence it appears to the writer that for the present, at least, Stenopsylla should be considered as synonymous with Doratopsylla. In regard to *D. antiquorum* Rothschild and the new species to be described in this paper there exists no good reason for keeping them in Doratopsylla hence a new genus is here proposed for their reception.

GENUS ADORATOPSYLLA NEW GENUS

Labial palpi four-segmented; genal comb with four spines; fifth tarsal segment of all tarsi with four lateral pairs of plantar bristles in addition to an inner pair between the first lateral pair; frons rounded; pygidium convex; antepygidial setae two, in addition to a minute hair; process of male clasper not divided into an anterior and posterior lobe; movable finger inflated posteriorly.

Type species.—*Adoratopsylla bisetosa,* new species.

Besides the type species this genus includes, as already indicated, *Doratopsylla antiquorum* Rothschild.

Adoratopsylla bisetosa, NEW SPECIES

Female.—Head with front unevenly rounded and genal comb extending all the way to the closed antennal fossa. There is a slightly curved, subfrontal row of five subequal setae. Behind this row and parallel to it is a second row of setae as follows: Upper seta of row large and situated near antennal fossa, next two setae very small and situated somewhat together, fourth seta very large, extending beyond lower margin of gena, fifth and lowest seta about one-third as long as the fourth one. Behind this second row of setae there are a few other minute setae and a very large one (ocular?) situated in front and above the ocular area. Number of spines in pronotal comb 16 to 18. Upper antepygidial seta about a fourth longer than the lower; above it is a minute hair or seta which is only seen by the aid of higher magnifica-

tions. Seventh abdominal tergite without notch, its ventral setae as follows: A postero-dorsal, submarginal row of two very large, subequal setae; in front of this row a parallel row of three subequal setae about one-third as long as the posterior ones; ventrally parallel to the ventral margin of tergite a row of two large, subequal setae, of the same size as the posterior row. Receptaculum seminis with an irregular oblong head, about twice as long as broad and an inflated, club-shaped tail which is about three-fifths as long as the head.

Length, 2.5 mm.; height, 0.9 mm.

Male.—Head broadly and evenly rounded in front, with frontal tubercle distinct. Chaetotaxy of head similar to that of female but the front row situated back from the margin of the frons; lower seta of second row longer than in the female. Antennal fossa open. Number of spines in pronotal comb the same as in the female. Clasper of male with a long, simple, sled-runner type of manubrium; dorsal process large, broad, with out-curving margins both in front and behind; bearing two very large setae considerably below the apex along the anterior margin, also some small setae. Movable finger very broad, being over a third as broad as long, with a subapical pair of small short spines, an apical seta which is short and directed backward, below this apical seta along the posterior margin is a much larger seta followed by one of about the same size as the apical one. In addition the movable finger has an inner subapical seta and several very short setae along its front margin. Ninth sternite exceedingly long and slender, of about uniform width, and bearing three ventral, subapical setae all of about the same size.

Length, 1.8 mm.; height, 0.6 mm.

Type host and type locality.—A marsupial, "coro," *Monodelphis brevicaudata*, Rio Branco, Santa Maria, Brazil.

Type slide.—Cat. No. 27886, U.S.N.M.

Described from three females and three males taken from "coro," *Monodelphis brevicaudata*, September 8, 1924, Rio Branco, Santa Maria, Brazil by the Harvard School of Tropical Medicine expedition to Brazil.

This species differs from its congener in having the posterior edge of the dorsal process of the male clasper outwardly rounded instead of emarginate, in having only two large setae on this dorsal process instead of three, and in having them situated along the anterior margin instead of apically. Also the three large setae on the ninth sternite of the male are situated ventrally and subapically instead of being situated apically as in *antiquorum*.

The remaining five species of Doratopsylla may be separated as follows:

Key to the Species of Doratopsylla (s. str.)

1. Anterior lobe of dorsal process of male clasper very long and bearing distally two large similar setae.....2.

Anterior lobe of dorsal process of male clasper very low and broad and bearing distally three setae one of which is enlarged.....3.

2. Genal comb extending all the way up to the antennal fossa, so that the last spine conceals the genal process.....*D. intermedia* Wagner.
   Genal comb not extending to the antennal fossa, so that the genal process is visible above the last spine.....*D. curvata* Rothschild.
3. Seventh abdominal sternite of female with a deep notch on its posterior margin.....*D. dasycnemus* Rothschild.
   Seventh abdominal sternite of female without notch on its posterior margin..4.
4. Process of clasper of male obliquely truncate; movable finger distinctly curved, more slender and broadly emarginate on distal half of anterior margin.....*D. blarinae* Fox.
   Process of clasper of male rounded, not obliquely truncate; movable finger almost straight, stouter and not emarginate on distal half of anterior margin.....*D. cuspis* Rothschild.

MULTIPLE ASSOCIATION IN *GREGARINA POLYMORPHA**

T. C. Nelson and J. A. Smith

During a laboratory period in parasitology early in November, 1924, the junior author discovered an association of three sporonts of *Gregarina polymorpha* from the gut of the common "mealworm" *Tenebrio molitor* (Fig. 2). Such an association had not been seen before in our laboratory and search of the literature revealed only one such case on record. In Miss Watson's (1916) monograph on gregarines there is pictured (Fig. 336) an association of three gregarines which is labelled "Unique association of three sporonts of *G. rigida,*" the author makes no further comment upon the unusual condition.

Systematic search of our tenebrio material was undertaken by both authors, weekly examinations being carried on from November 17, 1924, to January 15, 1925. Nine such triple associations and one quadruple association (Fig. 3) were discovered. In every instance the multiple associations were found in heavy infections of the gut. Three tenebrios yielded each two cases of multiple association, but in no instance were more than two such cases found in any one host. In Table 1 are given the dimensions of such of these associations as could be measured with a fair degree of accuracy. Owing to contraction of the gregarines on the one hand and pressure of the cover glass on the other these measurements must be considered as at best only approximate.

The tenebrios used in these examinations were obtained in two lots from a dealer in Mt. Joy, Pa. The first shipment was received about the first of October, 1924, and kept in corn meal at room temperature. The second shipment was received on November 20 and was kept in whole wheat flour, also at room temperature. Of the ten cases of multiple association found by us, seven were obtained from the first lot kept in corn meal and three from the second lot in whole wheat flour.

This unusual association of gregarines was discovered during the first cold weather in early November, during which time the jar containing the tenebrios was standing on the window sill subjected to relatively wide temperature variations. It was thought that the sudden fall in temperature might have stimulated association among the gregarines, hence tests were made by keeping lots of tenebrios at low

* From the Zoölogical Laboratory of Rutgers University.

(2-4 C.); at medium (room); and at constant (25 C.) temperatures. Examinations of these tenebrios over a period of three weeks failed to demonstrate any increase in either multiple or in normal associations as a result of exposure to high or to low temperatures.

The manner of union between the associated gregarines is of interest. Pressure brought to bear upon the protozoa through the coverglass demonstrated clearly that in cases of multiple association the union was quite as intimate as in the case of the normal association of two sporonts. Figure A illustrates the relations between the ectoplasm of the sporonts of Figure 1 as determined immediately after discovery. As will be noted from its figure the ectoplasm of the three associates was so intimately joined as to appear continuous. When first examined the endoplasm of the two posterior sporonts was con-

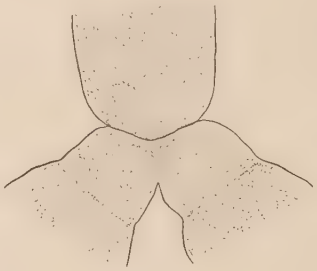

Figure A.—The region of contact between anterior and posterior sporonts shown in figure 1. Drawn with aid of camera lucida.

tinuous over a short region at the anterior end, but within a few minutes the epicyte at the point of union of the two gregarines pushed anteriorly, completely separating the sporonts.

MATURITY OF SPORONTS

In two out of the ten cases of multiple association found by us the sporonts were all mature as judged by the density of the protoplasm.

In specimens 4 and 5 of Table 1 it will be seen that one of the two posterior sporonts in each case exceeded the anterior sporont in length. In all other instances the posterior sporonts were immature, with semi-translucent protoplasm. Particularly is this true in specimen 6 (Fig. 3) in which three very immature sporonts were attached to one which was mature. As Miss Watson has shown, linkage of sporonts is no indication of maturity in the Gregarinidae. Interest centers about the possible fate of the nuclei should a triple association be successful in forming a cyst. In one specimen (Fig. 4), the anterior sporont was seen rotating in narrow circles dragging the two smaller posterior sporonts behind it, apparently in the attempt to accomplish encystment. After approximately 20 minutes the gregarine straightened out,

moved into a mass of intestinal contents and came to rest; it was then photographed.

The behavior of these sporonts during rotation was markedly different from that of two normal sporonts prior to encystment. In the latter case both sporonts enter actively into the process there being a true physiological as well as a morphological union between the organisms. In the rotation of the sporonts shown in Figure 4, the mature sporont behaved normally, bending sharply upon itself and describing narrow circles. The posterior sporonts, however, were entirely passive, made no bending movements whatever, and apparently took no part in the process. It is to be doubted therefore from the mode of encystment of this organism whether one active sporont dragging two quiescent individuals would be able to accomplish encystment. Cysts found during the course of our examinations were examined, but none

TABLE 1

	Size in Microns			
	Anterior Sporont	Posterior Sporont 1	Posterior Sporont 2	Posterior Sporont 3
1. Length	139.4	174.3	153.8	...
Width	61.5	61.5	51.3	...
2. Length	Not measured	149.7	151.7	...
Width	.....	118.9	98.4	...
3. Length	133.3	88.2	102.5	...
Width	51	24.6	24.6	...
4. Length	378	399	315	...
Width	113.4	109.2	117.6	....
5. Length	210	243.6	117.6	.....
Width	79.8	33.6	29.4	.....
6. Length	260	180	180	130
Width	102	39	32	32

showed evidence of having resulted from the union of three sporonts. Considering that only 4 out of 96 infected tenebrios showed cysts and that only 10 cases of multiple association were found among the thousands of gregarines examined, the chances of finding a multiple cyst, if such are formed, would be very slight.

DISCUSSION AND SUMMARY

Examination of 190 *Tenebrio molitor* between Nov. 17, 1924, and Jan. 15, 1925, showed 96 infected with *Gregarina polymorpha*. Twenty-five tenebrios were examined on April 8, 1925, all of which were infected. Among the 96 infected tenebrios studied during the fall and winter, ten cases of multiple association of the greparines were discovered. None was seen in the material examined in April. Seasonal variation in the abundance of gregarines in the Acrididae and in the Gryllidae was reported by Miss Watson, who found infection absent or light in spring and summer and heaviest in the autumn. In our

material, while no relative increase in the density of infection of single individuals was observed, the percentage of infection was found to increase from approximately 50% in the late fall to 100% in April.

Such an increase of parasites in tenebrios confined in large numbers under artificial conditions is to be expected. In nature the cold of winter would not only inhibit the reproduction of the gregarines but by preventing intermingling of the hosts would lessen the chances for spread of the infection. Under laboratory conditions with the tenebrios confined in large numbers in meal it is to be expected that through the accumulation of cysts in the meal every tenebrio would sooner or later become infected.

The ten examples of multiple association in *Gregarina polymorpha* found by us occurred in every case among very heavy infections. In some instances the gut was fairly swarming with the parasites throughout most of its length. It seems possible that under such conditions of crowding there might be competition for place of attachment to the wall of the gut, resulting in two or more immature specimens attaching to the posterior end of a mature sporont, as in Figure 3. Such an attachment would serve the immature specimens merely as a means of holding on, but it might be followed by rotation of the anterior sporont as already noted and even perhaps encystment, provided the posterior sporonts were mature and took an active part in the process. The problem is interesting not only from its bearing upon the fate of the nuclei in case three gregarines entered a cyst, but on account of the light which it might throw upon the beginning of associations among protozoa. Colonial protozoa are the result of failure of the cells to separate following division, but that aggregation of isolated cells might also result in the formation of colonies would seem to be at least a possibility.

Paper Cited

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Explanation of Plate

Fig. 1.—Three mature sporonts of *G. polymorpha* photographed while alive. Association No. 4 of Table 1.

Fig. 2.—One mature, two nearly mature, sporonts of *G. polymorpha*. Stained with alcoholic solution of eosin.

Fig. 3.—One mature, three quite immature sporonts of *G. polymorpha* photographed while alive. Association No. 6 of Table 1.

Fig. 4.—One mature, two nearly mature sporonts of *G. polymorpha* photographed while alive. Anterior end of mature sporont buried in intestinal contents of host. Association No. 2 of Table 1.

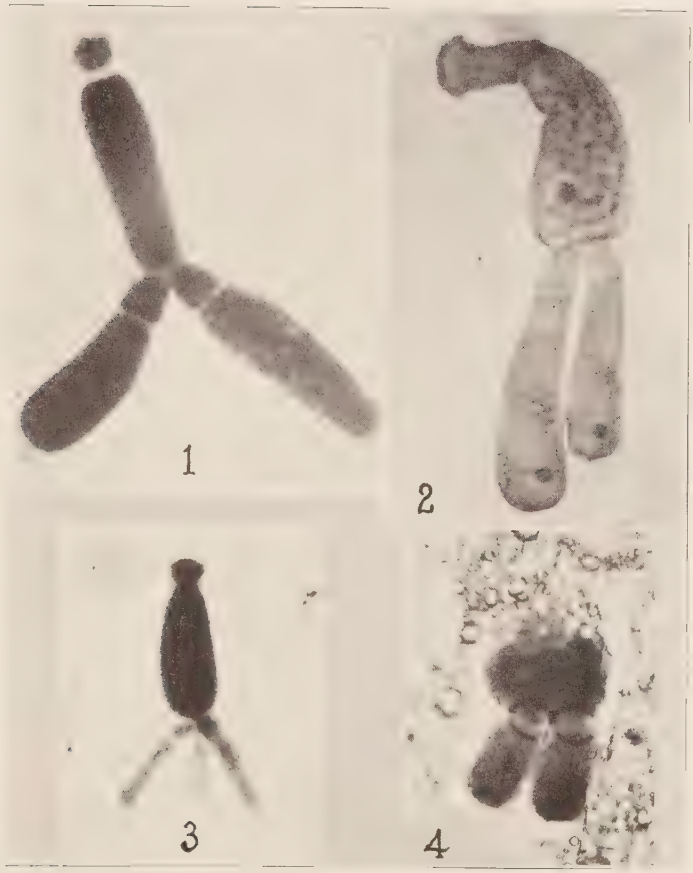

PLATE VI

PRELIMINARY NOTES ON A TREMATODE WITH ANUS

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In 1908 Leiper observed that in *Balfouria monogama* the intestinal ceca, which usually terminate blindly in other trematodes, opened into the excretory vesicle, and his observation has been confirmed by Odhner (1910), who found a similar communication also in another allied species, *Chaunocephalus ferox* (Rud.), as well as in a distant genus of fish trematodes, Haplocladus. In my collection of fish trematodes, I have found out two remarkable species which have a true anus on the ventral surface near the posterior end of the body. The digestive canal presents the usual condition, i. e., it is bifurcated, but the ceca communicate with each other behind, forming an intestinal arc, and this is followed by a short unpaired canal opening on the ventral side at a short distance from the posterior end of the body, and independently of the excretory vesicle, which opens at the posterior extremity of the body. The principal characteristics of these worms are as follows.

Opecoelus sphæricus nov. gen., nov. sp.

Body elongated, cylindrical, posterior part slightly flattened dorso-ventrally. When alive, reddish or creamy. Length 4.35 to 8.25 mm., width 0.35 to 0.95 mm. Cuticula smooth. Oral sucker subterminal, 0.18 to 0.37 mm. in diameter. Acetabulum at anterior part of second sixth of body, on a short pedicle, 0.20 to 0.37 mm. in diameter, with six finger-like protuberances at margin. Mouth and pharynx separated by tubular short prepharynx, 0.04 to 0.22 mm. long. Pharynx slightly elongated 0.12 to 0.20 by 0.13 to 0.22 mm. Esophagus 0.14 to 0.26 mm. in length, extending from pharynx to anterior level of acetabulum where it bifurcates. Intestinal ceca extending straight to near posterior end of body, where they unite and open on the ventral surface by an anal tube, 0.14 to 0.16 mm. in front of the posterior extremity.

Testes globular to elliptical, in median line, at anterior part of posterior half of body, one behind the other, separated from each other by a space equal to their own diameter or radius. Genital pore lateral, a little anterior to intestinal bifurcation. Cirrus pouch pear-shaped, containing a somewhat coiled short vesicula seminalis interna, globular pars prostatica, granular prostate gland and muscular cirrus; pouch left and anterior to intestinal bifurcation, succeeded by a long vesicula seminalis externa.

Ovary trilobed, median, in front of anterior testis, slightly separated. Shell gland diffuse, without definite outline, anterior to ovary. Laurer's canal present. Seminal receptacle absent. Uterine coil loose, occupying intercecal zone between ovary and genital pore; its initial part forms a receptaculum seminis uterinum, with numerous spermatozoa. Ova not numerous, oval, 70 to 71μ in length, 44 to 46μ in width. Vitellarium voluminous, follicles small, masses continuous, in lateral areas, extending from level of second sixth of body length to extreme posterior end of body; in post-testicular portion they coalesce across the median line. Transverse vitelline ducts and median vitelline reservoir at level of anterior margin of ovary.

Habitat.—In the intestine of *Leptocephalus myriaster* (Brevoort).

Locality.—Takamatsu, Kagawa Prefecture, Japan.

Opecoelus lobatus, n. sp.

Length 2.2 to 3.6 mm., breadth 0.50 to 0.51 mm., cuticula smooth. Oral sucker 0.16 to 0.17 mm. in diameter; acetabulum 0.24 to 0.27 mm. in diameter, on a short pedicle, with six finger-like protuberances at margin, situated a quarter of the body length from the anterior end. Pharynx 0.08 by 0.10 mm. Intestine as in *Opecoelus sphæricus.* Testes irregularly lobed, at anterior part of posterior half of body, almost directly tandem. Ovary slightly elongated transversely, trilobed, anterior to testes. Vitellarium extending from posterior level of acetabulum to posterior end of body, with irregular empty spaces. Uterus between ovary and genital pore. Ova 60 to 70 by 40 to 44μ.

Habitat.—In the intestine of *Parapristipoma trilineatum* (Thunberg).

Locality.—Choshi, Ibaragi Prefecture, Japan.

Opecoelus bears a very close resemblance to Coitocoecum Nicoll 1916, but differs from it in the presence of the anus and the marginal protuberances of the acetabulum. These two genera superficially resemble the Allocreadiidae, but the absence of the receptaculum seminis and the posterior communication of the intestinal ceca appear to exclude them from that family. In the male reproductive organs Nicoll notes the absence of the cirrus pouch in *Coitocoecum gymnophallum* as a remarkable characteristic separating Coitocoecum from Allocreadiidae, but that appears to me to be of no great moment. I have two undescribed species of Coitocoecum from *Mogurnda obscura* Temminck and Schlegel and *Tridentiger obscurus* Temminck and Schlegel, one of which has a faintly developed, flattened epithelial cirrus pouch and the other a muscular one, but in both Coitocoecum and Opecoelus the main part of the vesicula seminalis lies outside of the cirrus pouch while in the Allocreadiidae it is entirely enclosed by

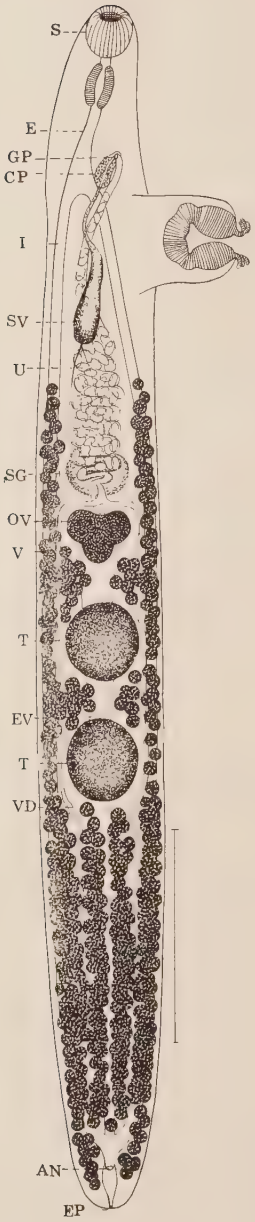

Figure 1

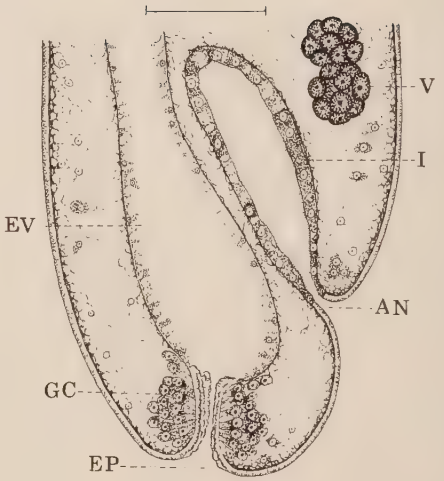

Figure 2

PLATE VII

the pouch. Except as regards the anal canal and anus, the two genera agree in all important structures, i. e., reproductive organs, excretory system and digestive organs. The anal canal and anus of Opecoelus is probably derived from the posterior intestinal arc of Coitocoecum by its backward prolongation and the formation of a pore. It seems, therefore, advisable to establish a new family to receive them, with the following provisional definition.

OPECOELIDAE nov. fam.

Worms of small to submedium size, with a thick, almost cylindrical body. Cuticula smooth. Oral aperture ventro-terminal or subterminal. Acetabulum slightly or considerably pre-equatorial. Acetabulum larger than oral sucker. Prepharynx, pharynx and esophagus present. Intestinal limbs unite at posterior end of body. Anus present or not. Genital pore preacetabular, lateral. Testes postacetabular, postuterine, postovarian, intercecal. Cirrus pouch present or not; vesicula seminalis not entirely enclosed. Ovary postacetabular, postuterine, pretesticular, and intercecal, median to submedian. Laurer's canal present; receptaculum seminis absent. Uterus transversely coiled, pretesticular, preovarian, intercecal. Vitellaria, with small follicles, lateral, uniting behind the testes. Parasites of fishes.

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Explanation of Plate VII

Fig. 1.—*Opecoelus sphæricus* ventral view. ×27. *AN*, anus; *CP*, cirrus pouch; *EP*, excretory pore; *EV*, excretory vesicle; *GP*, genital poré; *I*, intestine; *OV*, ovary; *E*, esophagus; *S*, oral sucker; *SG*, shell gland; *SV*, seminal vesicle; *T*, testis; *U*, uterus; *V*, vitellaria; *VD*, yolk duct.

Line at right of figure indicates 1 mm. in length.

Fig. 2.—Sagittal section of posterior end of body. Line at top 0.1 mm. long.

OPHTHALMOMYIASIS IN MAN DUE TO *CEPHALOMYIA* (*OESTRUS*) *OVIS* (LINN.)

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That the larvae of the common head-maggot fly of sheep, *Cephalomyia* (*Oestrus*) *ovis* (Linn.), may cause ocular myiasis in man is frequently mentioned in publications dealing with medical entomology, but references to specific cases occurring in this country appear to be very rare. This species enjoys a wide range and is fairly abundant in many parts; hence it would appear that attacks on man should be more common than is apparently the case, unless there has been a general neglect in reporting such attacks.

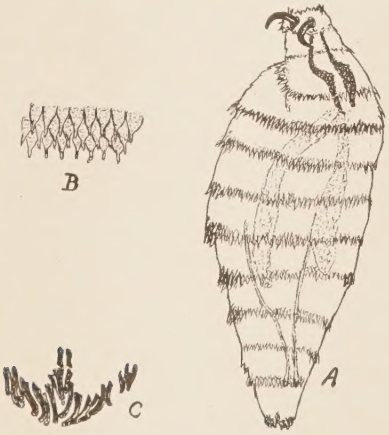
Ophthalmomyiasis of man traceable to *Cephalomyia ovis* is reported to be of rather frequent occurrence in Europe and in northern Africa. Portchinsky (1913) reports a number of cases occurring in Russia and describes the first stage larva of *Cephalomyia* (*Oestrus*) *ovis* (Linn.) as well as that of *Rhinoestrus purpureus* Br. which it resembles very closely. Larrousse (1921) reports a case from the region of Paris and includes a number of valuable illustrations as well as a review of literature on the subject. Gabrielides and Guiart (1922) cite a case from Constantinople in which fourteen first-stage larvae of this species were removed from the eye of a shepherd.

In a later publication Larrousse (1924) records a case of seven larvae of *Cephalomyia* (*Oestrus*) *ovis* being extracted from the eye of a person in charge of sheep in Indre, France. Pierce, in his Sanitary Entomology, states that, "Probably the most important species of this group is *Rhinoestrus purpureus* Brauer, which is a very common parasite of the horse in Russia, Hungary and Italy. This form is also responsible for cases of myiasis in the eyes of man, the attack apparently being similar to that of *Oestrus ovis*. Horses are infested by the flies which deposit larvae in the nose or eyes."

Under date of January 28, 1925, two specimens each measuring about one millimeter in length were sent to the writer from Honolulu for identification with the statement that they were taken from the eye of a patient by Dr. R. Faus, a third specimen being lost. The specimens were identified as first-stage larvae of *Cephalomyia* (*Oestrus*) *ovis* (Linn.). The larvae had been removed from the eye of the patient, December 5, 1924, the irritation being first observed two days previously. The patient's occupation was given as laborer in a warehouse of a chemical concern, and the irritation was noticed during the evening, after

dust from a keg of chemical compound in the warehouse had been accidentally blown into the left eye. The attending physician reported that the three larvae were buried in the sclera and were extremely adherent to the conjunctiva, causing acute conjunctivitis, lachrymation, ulceration and neurosis. No information was available as to whether the patient had been near sheep, goats or deer just prior to the infection. Professor D. L. Crawford of the University of Honolulu states that the species of Oestrid in question occurs in the vicinity of Honolulu but is not at all common.

A brief résumé of the habits and life history of *Cephalomyia ovis* will be helpful in understanding the behavior of this species in relation to man. The adult female fly, slightly more than half the size of a honey bee which it resembles somewhat, is yellowish brown in color and deposits living young. Deposition of young is accomplished while on



the wing by striking the nostril of the victim or by dashing into the eye. Under temperate climatic conditions young may be deposited over a period of several months from late spring to late summer. The case reported in this paper indicates that the fly is active probably throughout the year in the vicinity of Honolulu. The larvae thus deposited measure about one millimeter in length, are very spiny, possessing a pair of relatively large hooklets at the anterior end of the body (Text Fig. A), and are quite active. Migration upward into the nasal sinuses is immediately begun and with the rapid growth of the larvae the sinuses may soon become considerably clogged. Full growth is usually reached by the following spring when the larvae show a length of from 25 to 30 mm. When full growth is reached, the larvae wriggle their way out of the nostrils fall to the ground and pupate, the flies emerging from the pupa in from three to six weeks.

In the case of a person receiving the larvae in the eye there would be a greater probability that they would be removed than in the case of a sheep or other animal. In either case a route to the nasal sinuses, the normal lodging place, via the lachrymal ducts would appear to be open. This route of travel is probably seldom if ever taken.

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BOOK REVIEWS

DIE TIERSCHEN PARASITEN DES MENSCHEN. ERSTER TEIL: NATURGESCHICHTE DER TIERISCHEN PARASITEN DES MENSCHEN. By DR. MAX BRAUN. 608 pp., 416 text figs. Curt Kabitzsch, Leipzig.

The new (sixth) edition of this well known and highly prized work bears abundant evidence of the energy and intellectual vigor of the author. In the 42 years since the appearance of the first edition this work has grown from 238 to 608 pages of text and from 72 to 416 figures. In this latest edition one finds an increase of 10 per cent. over the fifth, with considerable further gain in space by virtue of the highly condensed style in printing references to literature and the abundant use of fine type in the general text. There are also 38 new and admirable illustrations. But this increase in size does not by any means give the measure of the revision. New material is found everywhere; sometimes in phrases or sentences and again in paragraphs or pages. In truth, the entire subject has been worked over and brought up to the level of present knowledge. One gets a vivid picture of the extent of recent research in this field by the multitude of new references and of textual changes involved in this thoro revision of a work that was brought up to date only ten years ago.

In the section on Protozoa almost every page shows more or less change from the earlier edition and the critical interpretations of new relations expressed by the author are frequent and valuable. Thus, among the amoebae names have been somewhat changed, descriptions made over and new species intercalated. Among the Flagellata the description of Chilomastix is almost entirely new; Lamblia has been entirely revised, as also Eimeria and Haemogregarina. In the Trematoda data are introduced on the development of Fasciolopsis and Paragonimus on *Echinostoma perfoliatum, Artyfechinostoma sufrartyfex* of Clayton Lane and *Euparyphium jassyense*. Still more extensive additions and changes have been made in the account of the blood flukes (Schistosoma) to bring it in line with the many important recent studies from Leiper to Faust. Braun criticises the use of the species name *Dicrocoelium dendriticum* which, as Braun indicates in this footnote, Odhner has shown is undoubtedly an error. And yet if such be the case why should Rudolphi's name be cited as a synonym? Incidentally the citation here of Stiles and Hassall as 1809 is one of the very few misprints noticed.

The changes in the section on Cestoda include many minor items under various species and more extensive additions to the life history of *Dibothriocephalus latus, D. mansoni,* and Hymenolepis. Some doubtful species are more fully discussed and others have been relegated to synonomy, all for what appear to be good and sufficient reasons. Among the Nematoda the account of the Filariae has been extensively revised. The family of the Thelaziidae appears for the first time, as does Gongylonema. The account of the development of *Ascaris lumbricoides* has been entirely rewritten to include the important recent studies on that topic. Much other new material has been added at various points.

Even among the Arthropoda, the careful scrutiny of the author has found a long series of small items which are incorporated in place. Larger matters are also cared for, such as the recently greatly extended evidence of the rôle of groups like the ticks and lice in the transmission of parasitic diseases. Here as elsewhere, much that is new came out of studies due to war conditions and many reports included by Braun give data which have not hitherto been in a form available to workers outside of Germany.

As regards nomenclature Braun is clearly a conservative. The preface states that he has refrained from making changes in names in order that ultimately stability may be achieved in the designation of species, as this is more important than strict application of the laws of nomenclature. All will agree most heartily with his desire; yet it is questionable whether the position of Braun is in fact calculated to achieve the result sought. At several points he suggests instability as when he lists Dioctophyme 1802 as a synonym of Eustrongylus 1851. It is difficult also to understand his use among Protozoa (p. 89) of the family name Distomatidae. His own pupil, Lühe, demonstrated that Dibothriocephalus was only a synonym of Diphyllobothrium, but the latter name is not even noted in Braun's text.

The list of references which despite condensed style in printing occupies more than 100 pages at the end of the book has been brought up to date and furnishes a most valuable record of recent continental studies in parasitology.

The revision is a distinct success and the work will continue to be one of the few absolutely indispensable books in the field of parasitology.

The Society for Tropical Therapy has published a commemorative number on the occasion of the dedication of its Institute at Leiden, Holland. Papers on amebic dysentery, the seat-worm, larvae of Dermatobia and *Metagonimus yokagawi* in the Dutch East Indies contain material of especial interest to the parasitologist. To a nation having large tropical colonies and an extensive maritime trade like Holland this new institute is sure to render great service and its work will be of equal significance to other nations also.

The *Proceedings of the International Conference on Health Problems in Tropical America,* held at Kingston, Jamaica, B.W.I., July 22 to August 1, 1924, have been printed by the United Fruit Company. The record forms an attractive and impressive volume of 1010 pages with 16 plates of which 5 are colored. The numerous papers which cover an extremely wide range of topics, tho many of them bear directly upon parasitology, cannot even be summarized here. The conference was held on the invitation of the officials of the United Fruit Company and the results, while of marked value to its work, constitute a significant contribution to tropical medicine and parasitology for the entire world.

A Monograph of the Tetraphyllidea has recently appeared in the *Memoirs of the Liverpool School of Tropical Medicine* from the pen of T. Southwell. It deals with a little known and much confused group in truly monographic fashion. By virtue of long continued investigations in India the author had unusual opportunities for studying these worms and has done a fine piece of work in interpreting the confused records of earlier students and in presenting a clear and well founded system for the group.

The Revista Yucateca de Dermatologia y Parasitologia is a new quarterly of which the first number appeared last May. It contains under the heading of notes on parasites 18 original microphotographs of Oosporaceae with explanatory text.

It is with deep regret, that we record the accidental death on May 20 of Dr. Samuel Taylor Darling. At the time Dr. Darling was traveling in Syria with the Malaria Commission of the Health Section of the League of Nations. As a contributor to the Journal and a cordial supporter of its work since the start, his loss will be felt both officially and personally. A tribute to his work will appear in an early number.